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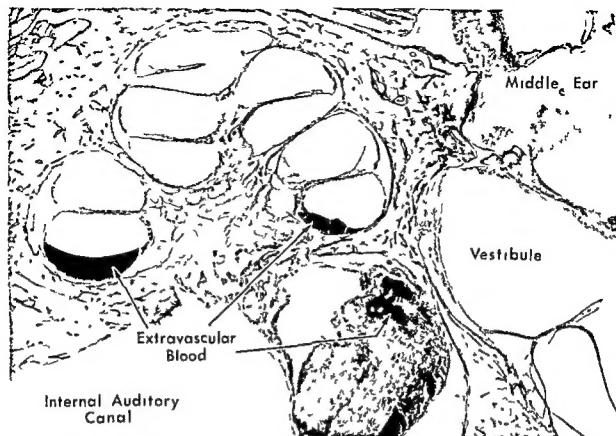


Fig 1 View from the right ear of a 44-year-old male who died 2 weeks after experiencing a spontaneous subarachnoid hemorrhage. Extravascular blood is seen

in the internal auditory canal and in the scala tympani of the basal turns of both ears. The cochlear aqueducts of both ears are patent and contain blood.

Most of the ears in which the cochlear aqueducts were blocked he found displacement of Reissner's membrane toward the scala vestibuli. He also reported finding a glassy precipitate in the scala tympani in these ears. He reasoned that the displacement of Reissner's membrane toward the scala vestibuli was due to a decrease in the volume of perilymphatic fluid and a compensatory increase in endolymphatic fluid to take up the space. He concluded on this basis that perilymphatic fluid was derived from cerebrospinal fluid which passed through the cochlear aqueduct. Subsequently, Nakamura (1967) repeated this experiment with similar results.

Several other experiments, however, in which bone dust and bone wax was used to block the mid part of the duct, tend to refute the idea that blockage of the cochlear aque-

duct causes endolymphatic hydrops. Lindsay et al (1952) blocked the cochlear aqueduct of two sound conditioned cats and found no morphological changes in the inner ears or alterations in auditory acuity after survival times of 4 and 8 months. Schuknecht & Kimura (1953) blocked both the cochlear aqueducts and vestibular aqueducts in 2 cats and after short survival times found mild endolymphatic hydrops. In these animals it could not be determined, of course, whether the endolymphatic hydrops was the result of blockage of the cochlear aqueduct or of the vestibular aqueduct or both. Schuknecht & Sen (1963) blocked the cochlear aqueducts of cats and after survival times of 10 months found all to have normal membranous labyrinth on histological study.

In view of the discrepancies in the results

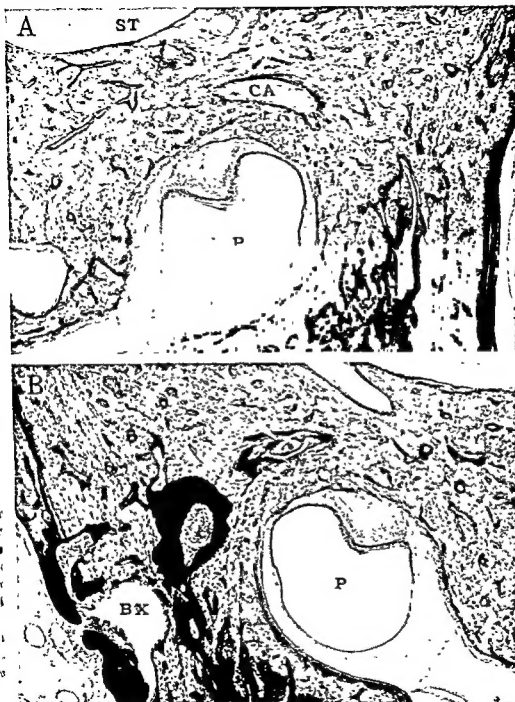


Fig. 2 (A) Photomicrograph showing the normal cochlear aqueduct (CA) situated between the posterior ampulla (P) and the scala tympani (ST) Guinea pig. (B) This photomicrograph shows the obliterated cochlear aqueduct (arrow) with new bone formation. The clear space (BX) represents the drilled area filled with bone wax which has been dissolved. Survival time, 5 months. P, posterior ampulla.

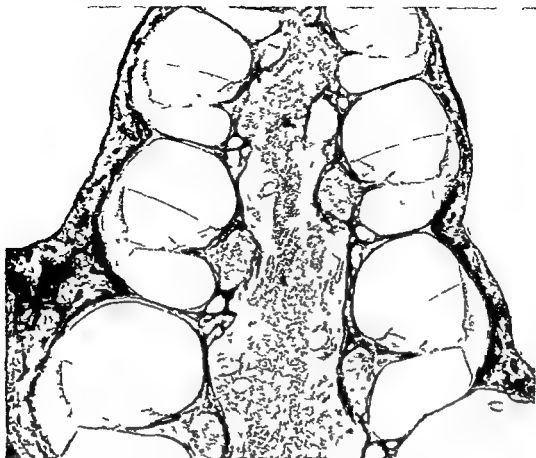


Fig 3 Photomicrograph of cochlea in which the cochlear aqueduct is obliterated. Note the normal position of Reissner's membrane and the normal organ

of Corti spiral ganglia and stria vascularis. Survival time 5 months. The same specimen is shown in Fig 2B. Guinea pig.

of these studies we repeated the experiment using guinea pigs. We used guinea pigs because of their proclivity for developing endolymphatic hydrops as evidenced in experiments on obstruction of the vestibular aqueduct. The cochlear aqueduct of the guinea pig is easy to obstruct because it is comparatively long narrows at its mid point and pursues a course separate from the inferior cochlear vein. In one group of animals we blocked the cochlear aqueducts only and in a second group we blocked both the cochlear and vestibular aqueducts at the same time. We reasoned that if the source of perilymph is interrupted by blocking the cochlear aqueduct the extent of hydrops resulting from the obstruction of the vestibular aqueduct should be enhanced.

MATERIALS AND METHODS

A total of 30 guinea pigs weighing 270 to 400 grams were used for this study. In the first group of 25 animals in which only the cochlear aqueducts were obliterated 21 were processed for light microscopy and 4 for electron microscopy. In each animal both ears were prepared for study, with one serving as the experimental ear and the other as a control. The specimens for electron microscopy (survival time 2-8 months) were fixed with 1% phosphate buffered osmium, embedded in Epon cut with an LKB ultratome stained with uranyl acetate and lead citrate and examined with a Siemens Elmiskop 1. The specimens for light microscopy (survival time 1-3 months 5 animals, 4-7 months 16 animals)



Fig 4 (A) Normal endolymphatic duct and sac (ES) adjacent to the common crus (C) S Sigmoid sinus Guinea pig (B) Obliterated vestibular aqueduct (arrow) Note the endolymphatic sac (ES) with the in-

creased rugose arrangement distal to the interrupted area S Sigmoid sinus C Common crus Survival time 5 months

were perfused with Heidenhain Susa em bedded in celloidin and cut serially at 20 μ m thickness

In the second group of 5 animals both the cochlear and vestibular aqueducts were obliterated during the same surgical procedure All of these animals were processed for light microscopic study (survival time 2–5 months)

The surgical method for blockage of the cochlear aqueduct was similar to the procedure we (Kimura & Schuknecht 1965) previously

used for blockage of the vestibular aqueduct After medial retraction of the cerebellum, the cranial aperture of the cochlear aqueduct was visualized A 4/0 cutting burr was used to drill into the cranial aperture of the aqueduct and follow it for a distance of about 1.5 mm at which point the lumen was very small The lumen of the aqueduct was then packed with bone dust and covered with bone wax The vestibular aqueduct was similarly blocked about 2 mm from the margin of the



5 Photomicrograph of cochlea in which both cochlear and vestibular aqueducts are obliterated an extensive hydrops and atrophy of the neuro-

sensory elements and the stria vascularis in the apical turns. The same specimen is shown in Fig 4B. Survival time 5 months Guinea pig

operculum. The opening on the skull dura was covered with gelfoam and the overlying soft tissues were closed in two layers.

FINDINGS

The cochlear aqueduct was successfully blocked in all ears prepared for light microscopic study (Fig 2) however four had to be eliminated from the study for the following reasons: two inadvertent arterial obstruction, one inadvertent venous obstruction and one bilateral (presumably pre-existing) atrophy of the organs of Corti.

None of the seventeen successfully operated ears showed endolymphatic hydrops (Fig 3). A slight displacement of Reissner's mem-

brane into the scala vestibuli in the basal turns near the internal aperture of the cochlear aqueduct was noted in three specimens; however the opposite control ears showed this same change. All sensory cells including the organ of Corti, maculae, utricle and saccule and cristae appeared normal. The other membranous structures including the stria vascularis, spiral ligament, limbus spiralis and tectorial membrane also appeared normal. The endolymphatic fluid appeared clear. The perilymphatic spaces of most experimental ears contained a fine granular precipitate which was present in only a few of the control ears.

The four ears prepared for electron microscopy were not evaluated as to the success of blockage though the cranial aperture was

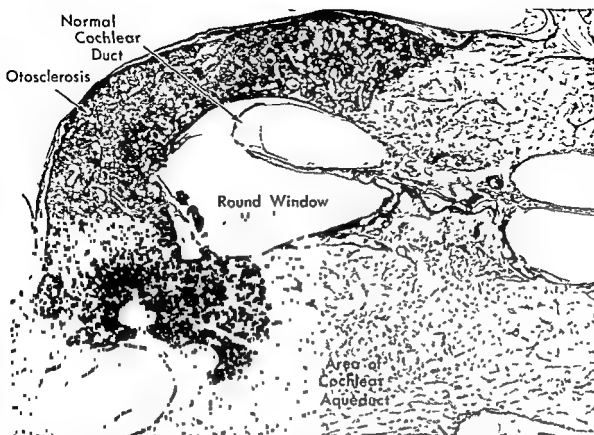


Fig 6 Photomicrograph of left temporal bone in a 69 year-old male with bilateral otosclerosis. Both ears show extensive otosclerotic involvement of the lateral parts of the bony labyrinths with obliteration of oval

and round windows and cochlear aqueducts. There was no evidence of endolymphatic hydrops in either ear.

covered by new bone. One of the specimens showed a bilateral atrophy of the outer sensory cells. The only change noted in other ears was the presence of vacuoles in the efferent nerve endings to the outer hair cells, but this change was also noted in the control ears. The afferent nerve endings showed no morphological change, nor did the subcuticular zones of the sensory cells. The stria vascularis appeared normal in all ears.

The experimental ears of the 5 animals in which both the cochlear and vestibular aqueducts were obliterated, exhibited endolymphatic hydrops involving the cochlear duct, saccule and utricle (Figs 4 and 5). In addition, there were degenerative changes in the membranous labyrinths of four of the five

ears. The ear of one animal (survival time: 2 months) showed a reduction in spiral ganglia in the fourth turn. A second animal (survival time: 2 months) exhibited atrophy of the spiral ganglia and outer hair cells in the third and fourth turns. A third (survival time: 5 months) presented loss of spiral ganglia and the outer sensory cells in the first, third and fourth turns. The stria vascularis was cystic and atrophic in a limited area of the first and fourth turns of this animal. In the experimental ear of the fourth animal (survival time: 5 months) there was a slight decrease in population of the spiral ganglia in the first and fourth turns, and of the outer hair cells in the second and fourth turns. In this ear the stria and spiral ligament were totally mis-

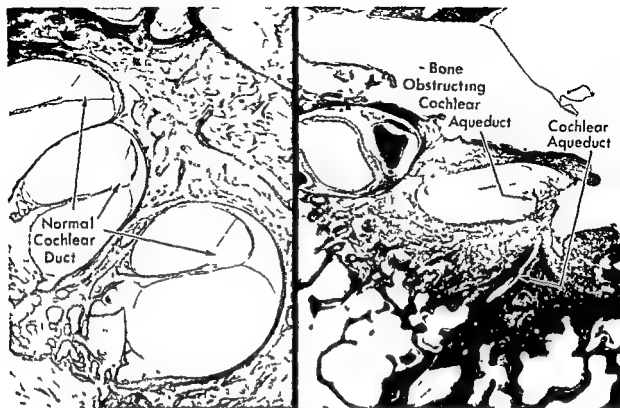


Fig 7 Photomicrograph from left ear of a 50-year old male who was profoundly deaf in both ears from early life. There is bone in the scala tympani of the basal turn which is obstructing the opening of

the cochlear aqueduct. There is atrophy of the organ of Corti throughout but the stria vascularis appears normal and there is no evidence of hydrops.

g in a small region of the basal turn. A few polymorphonuclear leucocytes were found in the perilymphatic space in this ear suggesting the presence of a mild inflammatory reaction. In general, these changes are of similar type and magnitude to those seen in previous experiments in guinea pigs in which only the vestibular aqueduct was obliterated (Kimura, 1967).

Light microscopic studies of the surgically obliterated cochlear aqueducts showed the lumens to be plugged by a thin layer of fibrous tissue upon which was superimposed a clear zone representing the area occupied by bone wax which was dissolved during the histological preparation, and finally by an irregular layer of new bone. In the gross specimens the bone wax appeared hardened when manipulated with a dissecting needle. It seemed to remain intact and surrounded by new bone

even after postoperative survival times of 5 months. There was some increase in fibrous tissue within the aqueducts, however, the tympanic orifices appeared identical to those of the control ears.

Examination of the obliterated vestibular aqueducts revealed a similar pattern of pathological change, that is fibrous tissue plugs at the cranial ends of the ducts with overlying layers of bone wax and irregular new bone.

Most of the animals of both groups showed no dysequilibrium or nystagmus following surgery. Slight nystagmus lasting for one day following surgery was noted in a few animals.

DISCUSSION

We believe the results of this experiment show conclusively that blockage of the cochlear aqueduct of the guinea pig causes no signifi-

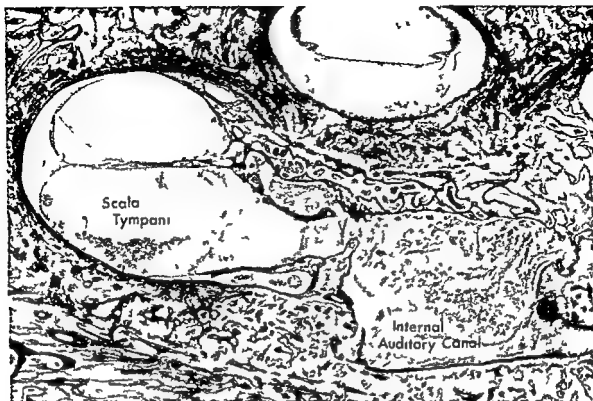


Fig 8 Photomicrograph from right ear of a 17 month-old male who died of acute pneumococcal meningitis. Pus cells are seen in the internal auditory canal and in the perilymphatic spaces of both

ears. In the right ear there is a direct communication between the internal auditory canal and the scala tympani of the basal turn.

significant morphological change in the inner ear. This observation is in agreement with the results of similar experiments by Lindsay et al (1952) and Schuknecht & Sefti (1963) who used cats and is in contradiction with the findings of Uyama (1933) who used rabbits and Nakamura (1967) who used guinea pigs. Although it is pure speculation we think it is possible that the hydrops produced by Uyama and Nakamura was of inflammatory etiology and caused either by surgical trauma or as a reaction to the sarcoma cells or the catgut suture.

The findings in the five ears in which both the cochlear aqueducts and vestibular aqueducts were blocked were no different from those we have observed previously from blockage of the vestibular aqueducts alone when postoperative survival times were the same.

Thus it appears that the endolymphatic hydrops and the atrophy of sensory and neural structures in these animals were the result of obstruction of the vestibular aqueducts and the consequent loss of function of the endolymphatic sacs and furthermore that these changes were not enhanced by blockage of the cochlear aqueduct.

Observations on histologically prepared human temporal bones further confirms the findings of our animal experiment. In our collection there are several specimens which show fibrous or osseous obliteration of the cochlear aqueducts without evidence of endolymphatic hydrops or other significant morphological change (Figs 6 and 7). In contradistinction to animals the cochlear aqueduct is only rarely widely patent in man (Schuknecht 1971) and usually it is very narrow. This ob-



Fig 1 Right ear of a 65 year-old woman who died 24 hours after a spontaneous subarachnoid hemorrhage. There is a massive infiltrate of fresh extravascular blood around the nerve trunks in the internal auditory canal. The blood cells have extended along the

nerve bundles to enter Rosenthal's canal and the fluid channels of the modiolus. The cochlear aqueduct is extremely small and no blood is seen beyond its mid portion, nor is there blood in the inner ear.

observation, also made by others (Waltner, 1948, Ritter & Lawrence, 1965) has already posed the question as to the functional importance of this structure.

Because of the chemical similarity of CSF and perilymph it seems to us rather probable that perilymph derives from the CSF and, possibly, from the fenestrated capillaries found among the modiolar cells (Kimura & Ota, 1973). It is obvious that perilymph is maintained in the presence of a blocked cochlear aqueduct. Our experimental results lead us to look for other routes by which the CSF may reach the inner ear. The most probable would seem to be via channels from the internal auditory canal and there are two routes by which this might occur. (1) There can be a

direct communication from the internal auditory canal to the scala tympani of the basilar turn. This route has been clearly observed in human ears and is the route by which infection may spread from the meninges to the inner ear (Schuknecht & Montandon, 1970). (See Fig 8) (2) In some human ears there is a communication from the subarachnoid space of the internal auditory canal to the spaces located in the modiolus. Schuknecht & Holden (1968) have observed that in some individuals these communications are large enough to accommodate erythrocytes (Fig 9). How CSF might reach the perilymphatic space from these modiolar spaces is not clear. The most probable route would seem to be along the perineural spaces of the auditory and ves-

tubular nerve fibers In the cochlea the CSF could then pass from the perineural spaces of the osseous spiral lamina through the canaliculae perforantes (Schuknecht et al, 1959, Lim, 1970, Tanaka et al, 1973) to reach the scala tympani

Another hypothesis is that perilymph is a blood filtrate arising directly from the vessels located in the spiral ligament and elsewhere (Altmann & Waltner, 1950, Kley, 1951, Hawkins, 1967) Several studies have shown that the spiral ligament is anatomically related to the perilymphatic space and has many channels communicating with it (Takahashi & Kimura, 1970)

ZUSAMMENFASSUNG

Es wurde versucht, beim Meerschweinchen nachzuweisen ob der Verschluss des Aquaeductus cochleae morphologische Veränderungen im Innenohr bewirken kann, und insbesondere ob es möglich ist, endolymphatischen Hydrops zu erzeugen Bei sieben Tieren, bei welchen der Aquaeductus cochleae mit Erfolg und ohne Beschädigung anderer Strukturen verschlossen wurde, waren bei der lichtmikroskopischen Untersuchung nach postoperativen Überlebenszeiten von ein bis sieben Monaten keine histologischen Veränderungen zu finden Auch durch Untersuchungen am Elektronenmikroskop Hessen sich keine feinen Strukturveränderungen nachweisen Bei fünf Versuchstieren mit postoperativen Überlebenszeiten von zwei bis fünf Monaten bei welchen sowohl der Aquaeductus cochleae als auch der Aquaeductus vestibuli verschlossen wurden, fanden wir endolymphatischen Hydrops und Degeneration der sensoriellen und neuronalen Strukturen von anatomisch gleichem Aspekt und Ausmass wie bei vorübergehenden Versuchen, bei welchen nur der Aquaeductus vestibuli blockiert wurde Es geht daraus hervor, dass die Funktion des Aquaeductus cochleae, der bei den meisten Säugetieren und manchmal beim Menschen eine weite Verbindung zwischen dem Liquor cerebrospinalis und der Perilymphe darstellt, ohne schädliche Auswirkungen auf das normale Innenohr ausgeschaltet werden kann Andere mögliche Ursprünge der Perilymphe sind die Liquorflussigkeit des Subarachnoiden Raums des inneren Gehörgangs und ein Filtrat aus dem Blut gefassten des Innenohres

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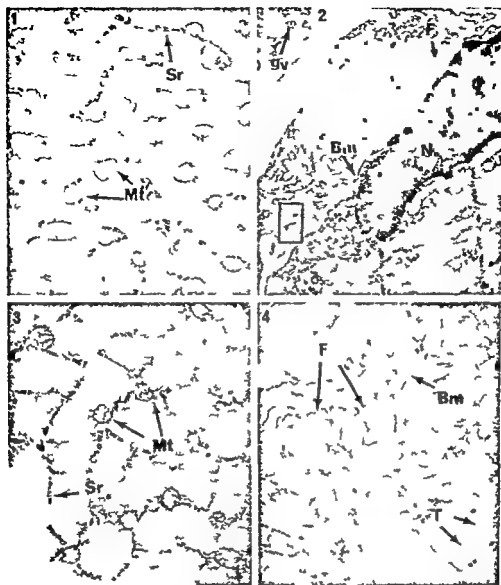


Fig 1 Transverse section of slow fiber showing poorly defined myofibrils, little sarcoplasmic reticulum (Sr) and numerous mitochondria (Mt) $\times 15\,000$

Fig 2 Nerve ending of slow fiber. A granule containing vesicle (enclosure) is shown at higher magnification in the inset (Gr). The junctional foldings (F) are very slight. Bm Basement membrane. N Nucleus. $\times 10\,500$ $\times 21\,000$

Fig 3 First type of twitch fiber in transverse section. Myofibrils are well delineated by sarcoplasmic reticulum (Sr). The mitochondria (Mt) are small and sparse. $\times 15\,000$

Fig 4 Nerve ending of first type of twitch fiber in longitudinal section showing well developed junctional foldings (F) on a raised sole area. T Transverse tubules. Bm Basement membrane. $\times 10\,500$

across the myofibril, an M line is clearly seen. The nerve endings of such fibers (Fig 4) have a large elevated sole area and well developed junctional foldings.

The second type of twitch fiber in transverse (Fig 6) and longitudinal (Fig 7) section displays myofibrils delineated by sarcoplasmic reticulum, moderate amounts of glyco-

gen and numerous mitochondria which tend to aggregate and form chains. Compared to the first type of twitch fiber, the transverse tubular system appears less elaborate, the 2 line is somewhat wider, the M line is less clearly discernible, and the junctional foldings at the nerve ending (Fig 7) appear less well developed.

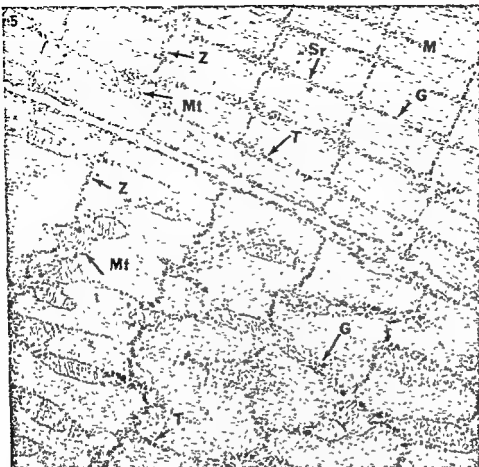


Fig 5. Longitudinal section of slow fiber (bottom) and first type of twitch fiber (top). The myofibrils of the slow fiber are not well delineated, while those of the twitch fiber are clearly separated. The Z line

is wider in the slow fiber compared with that of the twitch fiber. *Mt*, mitochondria; *Sr*, Sarcoplasmic reticulum; *T*, transverse tubules; *G*, Glycogen particles; *M*, *M* line $\times 15\,000$.

Laminated structures were observed under the cell membrane of a twitch fiber (Fig. 8). The dark lines show a periodicity of 1430–1860 Å. Multivesicular bodies appear associated with this laminated structure. The adjacent cellular space is devoid of myofibrils, but contains granular material, much glycogen, and many mitochondria some of which appear vacuolated, or broken.

DISCUSSION

The morphologically slow fiber observed in this study is essentially comparable to those reported in other middle ear muscles (Erulker

et al., 1964; Fernand & Hess, 1969; Hirayama & Daly, in preparation; Seiden, 1971). The mitochondrial distribution of this fiber, in terms of their considerable number and even dispersion, is similar to that of the slow fiber in tensor tympani of rabbit (Hirayama & Daly, in preparation). Reports on the middle ear muscles of other animals do not fully describe the mitochondrial distribution of the slow fibers (Erulker et al., 1964; Fernand & Hess, 1969; Seiden, 1971). The slow fibers of latissimus dorsi anterior muscle of chicken has numerous mitochondria (Hess, 1961) while slow fibers in extraocular muscle of rat have but few (Mayr, 1971). The wide Z line of this

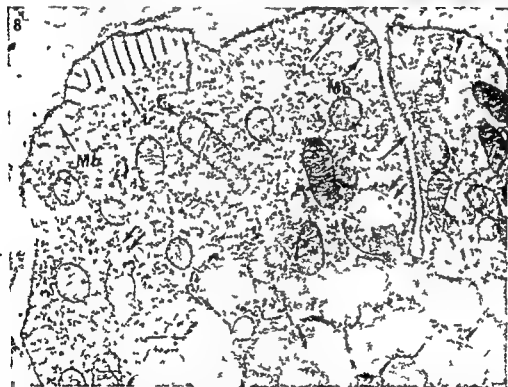
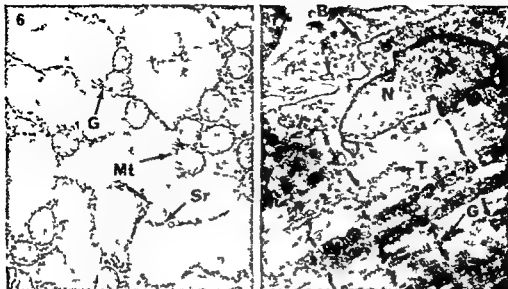


Fig 6 Second type of twitch fiber in transverse section showing myofibrils clearly separated by sarcoplasmic reticulum (Sr). The numerous large mitochondria (Mt) tend to form clusters. G Glycogen particles $\times 15\,000$.

Fig 7 Longitudinal section of second type of twitch fiber with nerve ending. The mitochondria (Mt) appear in clusters and long chains. The junctional foldings (F) are moderately developed. G Glycogen par-

ticles. T Transverse tubules. Bm Basement membrane. N Nucleus $\times 10\,500$.

Fig 8 Laminated structures (L) in twitch fiber associated with multicellular bodies (Mb). The adjacent cell portion contains numerous granular clusters, many glycogen granules and mitochondria, some of which are vacuolated or broken (double arrows). This atypical portion of the cell also shows a deep invagination (single arrow) of the cell membrane $\times 15\,000$.

slow fiber is consistent with reports of such fibers in amphibia (Page, 1965, Peachey & Huxley, 1962) and in rat extraocular muscle (Mayr, 1971). The presence of granule-containing vesicles in the terminal axon of this slow fiber agrees with descriptions of such fibers in extraocular muscle of cat (Cheng & Breinin, 1965).

The first and second types of twitch fibers and their associated nerve endings appear consistent, respectively, with descriptions of white and red types of fibers in mammalian skeletal muscle (Padykula & Gauthier, 1967). Previous reports of middle ear muscles described but one type of twitch fiber in each muscle (Erulkar et al., 1964; Fernand & Hess, 1969; Hirayama & Daly, in preparation; Seiden, 1971).

The laminated structure is comparable to that reported in the sensory epithelium of the inner ear (Friedmann, 1967; Hilding et al., 1967; Jahnke, 1969), the brain of cat (Morales & Duncan, 1966; Smith et al., 1964) and rat (Nauman & Wolfe, 1963) and extraocular muscle of man (Mukuno, 1966). Some investigators consider this structure to be associated with pathological changes, insofar as it was observed in the inner ear of patients with Meniere's disease (Friedmann, 1967) and in experimental ototoxic (Jahnke, 1969) or congenitally deaf animals (Hilding et al., 1967). It has also been suggested that they may serve as an anchoring device in extraocular muscle (Mukuno, 1966). In the present case, this structure is associated with an unusual appearance of the subsarcolemmal region, apparently far removed from the fibrillar structures.

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ZUSAMMENFASSUNG

Eine morphologisch „schwerfällige“ Muskelfaser wurde beobachtet sowie zwei Arten von „Zupffasern“. Die

„schwerfällige“ Muskelfaser hatte kaum getrennt Myofibrillen wenig sarkoplasmatisches Retikulum wenige querlaufende Tubulen und mehrere winzige Mitochondrien. Die beiden „Zupffasern“ sahen der weissen bzw. der roten Skelettmuskelfaser ähnlich. Ein blattartiges Gefüge wurde ebenfalls beobachtet.

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CHANGES IN AUDITORY LOCALIZATION DURING BODY TILT

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Abstract The induction of an error between a subject's true orientation and his registered orientation in relation to gravity results in auditory mislocalizations of a similar size and time course. The presence of visual cues prevents the development of errors in the interpretation of posture and, accordingly, prevents the development of errors in auditory localization as well. These observations are interpreted as evidence for a spatial reference system responsible for the maintenance of auditory and visual direction constancy. They demonstrate that where a subject hears a sound is dependent not only on the auditory cues in his ears but also on his registered orientation in relation to the gravitational force vector.

The constancy of auditory and visual direction is a familiar perceptual phenomenon. When an observer moves in relation to a stationary auditory or visual stimulus object he perceives himself as moving and not the stimulus. He knows also how much he has moved and where the stimulus object is with respect to his new position. If his performance were based solely upon the sensory cues from the stimulus source he could not determine whether he or the source had moved. In general, if the change in stimulus cues at the receptor surfaces represents a change of Δx in relative observer-source articulation, then Δx can result from movement of the observer by dis-

tance x and by movement of the source $x - \Delta x$, or the converse, where x may vary from zero to some finite distance dependent on the resolving characteristics of the receptor organs.

Consequently, in maintaining constancy of auditory and visual direction an observer must utilize, in addition to sensory cues, knowledge about the ongoing orientation of his eyes and ears. For example, in pointing to an external sound source, he must take into account the auditory cues from the sound source and the position of his head with respect to his trunk and hand. Since his ears are stationary in relation to his head, it is often thought that the observer can make judgments about the position of the sound source in relation to his head simply on the basis of the auditory cues without taking into account the orientation of his head.

That an observer can localize a sound source is in itself presumptive evidence for the existence of an internal reference system that permits him to relate the auditory cues at his ears to the moment to moment spatial orientation of his head and body. It is this neural reference system relating postural and sensory information that permits him to make two types of auditory judgments, head relative localizations (saying whether the sound is to the left or right of the median plane of the head) and more general body relative localizations

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(pointing to the sound, saying whether it is to the left or right of the torso, or left knee, etc.)

It seemed likely that if an error could be induced in the relating of the auditory cues as given at the ears to postural information about body orientation that errors in auditory localization would result. A central concern of the experiments to be described was to demonstrate that when a postural error is introduced not only "pointing localizations" but all head relative localizations to an ambient sound are affected. In other words it is being claimed that even when an observer is saying whether a sound source is to the left or right of the median plane of his head he is taking into account the perceived position of his head with respect to his trunk and with respect to the gravitational vertical, as well as the auditory cues from the sound source.

To test the validity of this assumption it was necessary to find a way to introduce an error between the observer's true head position and his registered head position. Several earlier experiments furnished a clue. Teuber & Liebert (1956) had found that a tilted subject in setting an ambient sound source to his median plane consistently places the source too far to the upward side, i.e., he "underestimates" the position of his median plane. By contrast, under identical tilt conditions, a subject asked to set a dichotically presented sound to his median plane hears the sound as centered only when his down ear is being given a considerable time advantage relative to his other ear (Teuber & Diamond 1956).

It had been noticed too that a blindfolded subject who has been tilted and then returned toward the upright considers himself as upright when he is still tilted somewhat in the original direction (Clark & Graybiel 1963, Fleishman 1953, Mann & Passey, 1951, Mann & Ray, 1956, Passey & Guedry 1949, Teuber & Mishkin 1954). This underestimation of tilt is a very strong one: after being tilted for a brief period of time the subject actually feels less tilted than he is.

The possibility arises that the underestimation of body tilt in blindfolded subjects is responsible for the auditory displacements observed by Teuber & Liebert (1956) and Teuber & Diamond (1956). If these factors are in fact related, then the magnitude of the auditory errors during body tilt should be directly proportional to the magnitude of the postural adaptations to tilt, and have similar time courses. Accordingly, these two experimental paradigms, when combined, furnish a technique for testing the hypothesis that a discrepancy between a subject's true orientation and his perceived or 'registered' body orientation leads to systematic errors in auditory direction.

An additional prediction is possible. When a blindfolded subject, after prolonged tilt, is returned to the upright his auditory localizations should exhibit aftereffects as a consequence of his feeling tilted past the vertical somewhat to the opposite side. How auditory localizations would be affected if the subject were tilted but not blindfolded is less readily predicted. In this situation the subject has veridical visual information concerning his orientation relative to gravity as well as misinformation from either vestibular or somatic sensory sources or both. In general, in situations involving a conflict between visual and postural cues the visual cues predominate (Hay et al., 1965, Lackner, 1970, Nielsen, 1963); consequently, it might be expected that the auditory localizations of a tilted subject permitted unrestricted vision would be veridical.

The experiments to be described systematically explored these issues as well as the distinction made by Teuber & Liebert (1956) and Teuber & Diamond (1956) that the errors in localization of ambient and dichotic sounds during body tilt are in opposite directions.

SUBJECTS

Twenty-four male MIT undergraduates were paid for their voluntary participation. Prior to being recruited each candidate's auditory

thresholds were measured with a Bekesy audiometer, to qualify for inclusion, his pulsed tone audiograms for both ears had to be within 5 dB of each other throughout the frequency range 100–10 000 Hz. In addition, no one with tonal lacunae or known vestibular defects was included. For every prospective subject meeting these requirements two failed

APPARATUS

Tilt chair

The tilt chair used in these experiments provided lateral tilts in three discrete steps, left or right, to a maximum of 28° . When a subject was being moved toward or away from the upright, the experimenter could read the tilt angle from a scale to the nearest $1/2^\circ$. An adjustable head cushion was mounted on the back of the chair and a metal slide supporting a vertical, 60° coronal arc of 12 inch radius was attached to its frame. A small carriage holding a single Grason-Stadler headphone that was used to deliver auditory stimuli during ambient sound localization tasks could be moved noiselessly on the metal arc. The "zero" of the calibrated arc corresponded to the median plane of a seated subject's head. The face of the earphone was mounted in the slide carriage perpendicular to the base of the chair, a pointer permitted readings of its position to the nearest $1/4^\circ$. An adjustable head holder and chin rest held the subject's head rigid during experimentation.

Sound generating equipment

Auditory clicks were generated by gating a white noise source for 1 msec, independent control of the duration and intensity of signals in two channels was possible. For dichotic presentations the interchannel separation could be varied continuously from simultaneity to 1 sec with either channel leading. For ambient presentations only one channel was used. A detailed description of the auditory apparatus is available in Lackner (1973).

AUDITORY PROCEDURES

Dichotic localization

Before auditory localization measurements were made, click detection thresholds were determined for each of a subject's ears so that the stimuli to be localized could be set at a constant level above his thresholds. During threshold measurements, the subject, wearing headphones, was instructed that he was being tested for the "softest" click that he could hear. His task was to raise his index finger and keep it raised as long as he could hear clicks in the ear being tested, to lower his finger when the clicks became inaudible, and, then, to raise it as soon as he again could detect the clicks. The clicks presented were 1 msec in duration, cycled once per second. Each threshold determination began with the clicks well above the subject's threshold and then click intensity was decreased in 1 dB steps until the subject again raised his finger, this complete cycle was repeated 12 times. The subject's threshold was defined as the average of the final 10 descending and 10 ascending settings. This procedure is modeled on techniques developed by Bekesy (1947) for automatic audiometry.

For the dichotic localization task, the subject was instructed that once each second he would hear a click inside his head somewhere between his ears. He was told to listen carefully and to signal "right" or "left" for every second click depending on whether he had heard it to the right or left of the center of his head. The subject indicated "right" or "left" by manipulating a silent micro-toggle switch which activated signal lights in sight of the experimenter.

A particular determination in a given experimental condition began with clicks presented from an intermediate position, one ear or the other leading by 100 or 200 μ sec, randomly selected. Click intensity in each ear was adjusted to 40 dB above absolute threshold. If the subject started off responding "left", his right ear was given a progressive time

advantage (≈ 5 microseconds/second) until he responded "right" three consecutive times, then his left ear was given the time advantage until three consecutive "left" judgments were obtained. This sequence was cycled back and forth 12 times and the mean of the last ten sequences (ten "left" and ten "right" values) was designated the "auditory midline".

This procedure was validated by comparing for 10 subjects auditory midlines obtained with this method with auditory midlines determined using the method of constant stimuli. The midlines obtained with the two techniques were not significantly different.

Ambient sound localization

For ambient localization, the subject was seated in the tilt chair with his head stabilized by the head holder, and the center of the arc carrying the sound source was placed 12 inches above his occipital pole. The sound source was set at the zero position (i.e., the subject's median plane) and click detection thresholds were determined with the subject listening with both ears, using the bracketing procedure described above. During subsequent auditory localization, click intensity was raised 40 dB above threshold.

For the ambient localization task, the subject was told that he would hear clicks coming from somewhere in the coronal plane of his head, once each second, and that he was to indicate by manipulating a toggle switch which way the sound source had to be moved to place it in the median plane of his head. The subject was allowed all the time he needed in setting the sound source.

Five separate localizations were obtained, each beginning from a different starting position. After a localization, the clicks were turned off while the sound source was moved to its next starting position. The five starting positions were employed in a counter-balanced order and included left (L) and right (R) placements (relative to the subject's median plane) of 20°L, 10°L, 0°, 10°R and 20°R.

The mean of the five localizations was designated the "auditory midline".

Experiment 1

Influence of body tilt on auditory localization

Measurements were made of the auditory localizations and perceived posture of tilted subjects to determine whether postural errors and auditory midlocalizations are correlated. Both dichotic and ambient localization conditions were included to see whether the direction of auditory displacement would be opposite for dichotic and ambient sounds, as suggested by the results of Teuber and his co-workers (Teuber & Diamond, 1956, Teuber & Liebert, 1956). This experiment was undertaken only after a control experiment failed to reveal differences in click detection thresholds during body tilt, thereby eliminating the possibility that changes in localization might result from peripheral factors.

Procedure. Two groups, each of 12 subjects, made auditory localizations under each of 6 tilt conditions, 28°L, 28°R, 16°L, 16°R, 6°L and 6°R. The tilt conditions were presented in a balanced design and were preceded and followed by auditory localizations with the subject upright. One group of subjects wore headphones and localized dichotic clicks, the other group localized ambient clicks. Each subject was instructed that following a tilt condition he would be returned toward the vertical and when his body felt perfectly upright (i.e. perpendicular to the floor) he was to tell the experimenter. The subject was permitted one correction.

After the instructions were completed the subject was fitted with opaque goggles and the experiment was initiated with testing in the first upright condition. In the tilt conditions, the subject was told in which direction he would be tilted, but not how far. After being tilted, the subject was kept in position for 3 min before testing began. Following return to his perceived upright, the subject was

moved to the next tilt position without being told whether he had been upright. Consequently, he never gained direct information about the extent or direction of his error in posture.

Results Errors of opposite sign occurred in the dichotic and ambient localizations of all subjects during tilt. The magnitudes of these displacements for a given tilt position were directly related to the postural adaptation effect occurring in that condition, the larger the adaptation to body tilt, the larger the auditory displacement. In addition, the size of the postural adaptation to tilt increased with larger tilt angles.

Fig 1 illustrates the relationship between dichotic auditory localization and adaptation to body tilt for the various tilt conditions, the auditory shifts are in the same direction as the body tilt (The 6°L and 6°R conditions are omitted because no significant changes either in auditory localization or perceived posture occurred in these conditions, $p > 0.05$). Fig 2 shows corresponding data for the localizations of the ambient source, here the auditory shifts are in the direction opposite the body tilt. The figures indicate the

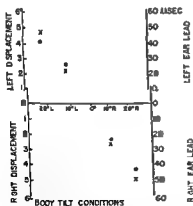


Fig 1 Relationship between postural adaptation and displacements in dichotic auditory localization. Filled circles (right ordinate) indicate the necessary lead times to one ear to recenter the sound image when subjects are tilted. X's (left ordinate) indicate the subjects' true orientation in relation to gravity when they perceive themselves as upright following completion of the experimental trial.

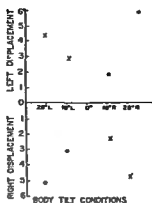


Fig 2 Relationship between postural adaptation and displacements in ambient sound localization. Filled circles (left ordinate) indicate the true locus of the sound source in relation to the body during the experimental trial when the sound is perceived to be in the subjective median plane. X's (left ordinate) indicate their true orientation in relation to gravity when subjects perceive themselves to be upright upon completion of the experimental trial.

auditory cues for which the stimuli are localized in the midline of the body.

The effect of starting position on the localizations of subjects performing dichotic localizations was slight, in all cases less than 10 μ sec. By contrast, the effects of starting position on the localizations of ambient sounds were much larger: 3.6°L and 3.9°R for starting positions of 20°L and 20°R, respectively, and 2.1°L and 2.4°R for 10°L and 10°R.

Comment *Experiment 1* The experimental observations confirm the reports of Teuber & Liebert (1956) and Teuber & Diamond (1956) that the errors in auditory localization made by blindfolded, tilted subjects are opposite in direction for ambient sounds and dichotically presented sounds. These findings also support the notion that auditory direction is dependent upon the registered orientation of the head in addition to the auditory cues at the ears.

Experiment 2

Time course of auditory displacement during body tilt

That the magnitudes of the subject's auditory displacements and postural adaptations during

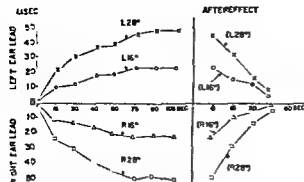


Fig 3 Changes in the localizations of dichotically presented sounds as a function of elapsed time during and after tilt of the body axis

tilt covary may be a coincidence unless they can be demonstrated to have similar time courses. Accordingly, the present experiment was designed to monitor over time these changes

Procedure The two groups of subjects from Expt 1 also participated in this experiment. Four tilt conditions preceded and followed by testing at the upright, were employed (in a balanced order) 28°R, 16°R, 16°L, and 28°L. Measurements of auditory localization were taken as soon as the blindfolded subject was tilted into position and were continued until the localization values had stabilized. The subject was then rapidly returned to the upright (~5° sec) and auditory testing was continued

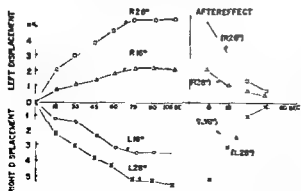


Fig 4 Changes in the localizations of ambient sounds as a function of elapsed time during and after tilt of the body axis.

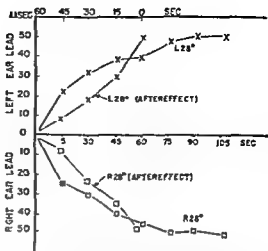


Fig 5 Comparison of the development of changes in dichotic auditory localization during body tilt with the dissipation of these changes upon return of the body to the gravitational vertical. The aftereffects are plotted in relation to the upper time base

until his localizations had again stabilized. This procedure was repeated for each tilt condition until all four conditions had been explored.

Results The results for the dichotic and ambient localization conditions are presented individually in Figs 3 and 4. Auditory displacements developed gradually over time reaching a maximum within 1 1/2 to 2 min. After 2 min, no further changes in auditory localization occurred. When a subject was returned to the upright, his auditory localizations rapidly returned to normal.

Fig 5 illustrates the development of the displacements in auditory localization during body tilt and their decay when the subject was returned to the upright; the decay is much more rapid than the initial development.

Comment Experiment 2 The pattern of these results is dependent on abnormal changes in registered posture requires that a subject when initially tilted rapidly comes to feel less tilted than he actually is and upon being returned to the upright experiences himself as being tilted somewhat to the opposite side.

Experiment 3

Time course of postural adaptation during body tilt

In conjunction with the results of Expt 2 this experiment should establish whether abnormal changes in registered posture are the determining factors in the auditory displacements that occur during tilt

Procedure The same subjects, tilt conditions (28°L , 28°R , 16°L , 16°R) and order of conditions were used as in Expt 2, although no auditory localizations were involved

Each subject was instructed that he would be tilted and held in position for varying periods after which he would be returned toward the vertical, he was to concentrate carefully and report when he again experienced himself to be upright. The experimenter then tilted the blindfolded subject into position for the appropriate time (15 sec, 30 sec, 45 sec, 60 sec, 75 sec, 90 sec, or 105 sec), returned him toward the upright, noted the value at which he reported himself upright, and returned him to the true upright. When the subject again reported himself upright, he signalled the experimenter who recorded the time elapsed from the subject's actual return to the upright. This procedure was repeated for each tilt condition.

Results The experimental results for both groups are presented in Fig 6. When compared with the findings of Expt 2, it is clear that the time course of the auditory displacements during body tilt and the time course of the postural adaptations are virtually identical. The length of time a subject is tilted before his auditory localizations stabilize is dependent on the time it takes for his postural adaptation to reach a maximum. The size of the final (end point) auditory localization displacements obtained in a given tilt condition in Expt 2 is not significantly different from the size of the adaptation effect to tilt observed in the comparable condition in Expt 3 ($p > 0.05$).

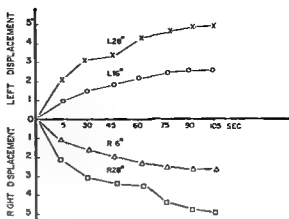


Fig 6 Development of postural adaptation as a function of body tilt and elapsed time. The ordinate specifies the angle of the body in relation to the gravitational vertical upon return to the perceived upright.

As they were returned toward the vertical, all subjects reported themselves to be upright when they were still tilted to the same side, upon reaching the true upright, they reported themselves to be tilted somewhat to the opposite side. The time elapsed after the subjects were returned to the upright before they reported feeling upright was not significantly different from the duration of the aftereffects in auditory localization found in Expt 2 ($p > 0.05$).

Comment, Experiment 3 Taken together the results of Experiments 1, 2, and 3 implicate postural adaptation effects as being responsible for the auditory displacements that occur during body tilt. Thus, a subject's auditory localizations are made with respect to his orientation as registered by his central nervous system even though his body may in fact be more tilted.

Experiment 4

Auditory localizations during body tilt of subjects with unrestricted vision

Ordinarily, vision is our most important sense, since most orientation and movement take place under visual guidance. Therefore, it is of interest to determine whether subjects per-

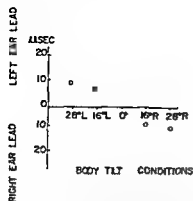


Fig 7 Changes in dichotic auditory localization as a function of body tilt in subjects with unrestricted vision. The ordinate indicates the necessary relative time advantage to one ear to produce a centered sound image.

mitted visual information concerning their true body orientation will show postural adaptation effects and auditory displacements during body tilt.

Procedure The experimental design was identical to that of Expt 1, except that subjects were permitted unrestricted vision, consequently, at all times they had information from an articulated visual environment about their true orientation relative to the vertical.

Only dichotic localization was used in this experiment, so that there would be no possibility of the subject seeing the sound source.

Results Fig 7 presents the experimental findings. Significant changes in auditory localization did not occur ($p > 0.05$).

Comment Experiment 4 The results suggest that the reference frame for orientation is set primarily by the visual input and that other modalities serve to establish the reference coordinates primarily in the absence of vision.

Experiment 5

Influence of prior visual information on auditory localization during body tilt

Although the presence of visual information leads to accurate auditory localizations dur-

ing tilt, it is important to determine when vision is restricted *after* the subject is in position, whether the subject will revert to the use of proprioceptive-vestibular tactile information about his body orientation or whether he will be able to "remember" his position (as specified by the prior visual information) and maintain veridical performance.

Procedure The design was similar to that of Expt 4 except that within each tilt condition a subject was blindfolded and immediately retested after his auditory localizations had been measured the first time. Then, the blindfold was removed and the subject with his vision unrestricted was returned to the upright, whereupon he closed his eyes and his dichotic auditory localizations were remeasured.

Results From Fig 8 it can be seen that as long as a subject's eyes were open, his auditory localizations remained stable and veridical, however, as soon as he closed his eyes, his auditory localizations assumed values closer to those found in experiments in which the subjects were blindfolded throughout. In the aftereffect condition, there were small, non-significant displacements in auditory localization.

Comment Experiment 5 This experiment demonstrates that as long as visual informa-

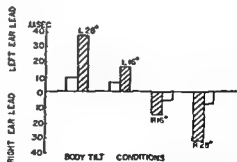


Fig 8 Changes in dichotic auditory localization after 3 min of body tilt with eyes open. Empty bars indicate localizations with eyes open, hatched bars indicate localizations with eyes closed. Subjects are tilted during the measurements.

tion is available concerning the true extent of body tilt it "dominates" other sensory inputs in aligning the spatial "reference frame" used in localization. When vision is excluded the remembered knowledge of true body orientation is not sufficient to maintain veridical localization and other sensory sources are progressively relied upon.

Experiment 6

Postural adaptation effects in tilted subjects with unrestricted vision

The pattern of results just described raised the question whether visual knowledge of "true" body orientation and nonveridical postural information from other modalities could be maintained simultaneously.

Procedure The design of Expt 6 is similar to that of Expt 3, except that a subject was tilted with his eyes open. He remained in his tilted position for three minutes, then was blindfolded and returned toward the upright. The experimenter noted the point at which the subject reported being upright, returned him to the true upright, and recorded the elapsed time before the subject again reported himself to be upright.

Results Subjects showed significant postural adaptation effects ($p < 0.05$) but of a much smaller size than those observed in experiments in which the subjects were blindfolded throughout the experimental procedure. The results are illustrated in Fig 9.

Comment Experiment 6. Even when a tilted subject has visual information concerning his body orientation small, postural adaptation effects are developing in the representation of his body orientation as specified by other sense modalities. If the visual information is removed the subject relies on vestibular or proprioceptive "misinformation" to assess his body orientation. It is clear, however, that the magnitude of the postural adaptation effects de-

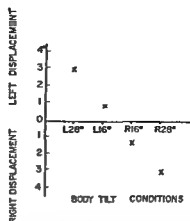


Fig 9 Magnitude of postural adaptation after 3 min of body tilt with eyes open. The ordinate indicates true body orientation when subjects perceive themselves as being upright upon being returned (with eyes closed) toward the gravitational vertical.

veloping in these other modalities is attenuated in the presence of visual input.

DISCUSSION

The ability to localize a sound in space requires a reference system by which the auditory cues at the ears are assigned to various external directions. In conjunction with information about the position of the head in relation to the trunk (and the head relative to gravity) this spatial reference system permits judgments about the direction of a sound source with respect to the head or torso. The experiments that have been described systematically investigated the interrelation between this reference system and positional information about body orientation.

Specific predictions were made concerning the interaction of postural and auditory cues in the establishment of auditory direction. In particular, it was predicted that changes in perceived posture should be reflected by changes in auditory direction of similar magnitude and time course, and, moreover, that in keeping with the findings of Teuber & Liebert (1956) and Teuber & Diamond (1956), the direction of auditory change should be opposite

for dichotic and ambient signals. All of these predictions were supported by the outcome of the experiments.

Although the observations of Teuber & Diamond (1956) and Teuber & Liebert (1956) that during body tilt the direction of displacement for ambient and dichotic sounds is opposite were replicated, the reason for this difference is unclear. An analogous situation seems to hold, however, for vision.

Roman et al (1963) and Warren et al (1963) have reported that the apparent displacements of afterimages and external visual targets during the 0-g (weightless) phase of parabolic flight are in opposite directions. During 0-g, as in the present paradigm involving body tilt, an abnormal change in perceived posture occurs and it is possible that the opposite signs of the displacements are related to whether the stimulus is interpreted as being 'anchored' to the subject and thus "moving" with the subject's changed interpretation of his position (e.g., dichotic clicks presented via headphones afterimages) or as being independent of his motion (e.g., ambient clicks, external visual targets). Additional experimentation is clearly necessary to refine this distinction, but can nevertheless be concluded on the basis of the present experiments that not only the auditory cues at his ears but also information about his orientation in space determine where a subject will hear a sound.

ACKNOWLEDGMENT

I thank Professor H. L. Teuber for his valuable comments and encouragement.

ZUSAMMENFASSUNG

Der Unterschied zwischen der wahren und der von einer Versuchsperson selbst wahrgenommenen Orientierung ergibt beim Hören Fehlokalisierungen ähnlichen Grossen und Zeitaussmasses. Gewisse Stützpunkte der visuellen Umwelt jedoch verhindern die Entwicklung von falschen Interpretationen der eigenen Körperhaltung und dadurch auch der auditorischen Lokalisierung. Diese Beobachtungen werden als Beweis für ein räumliches Referenzsystem angesehen, das für die Erhaltung einer Richtungskonstanz

beim Hören und Sehen verantwortlich ist. Sie zeigen weiter, dass die Position des Hörens nicht nur von den ans Ohr dringenden Lauten abhängt, sondern auch von der selbst wahrgenommenen eigenen Körperorientierung.

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EFFECT OF CONTRALATERAL BROAD BAND NOISE ON FREQUENCY DISCRIMINATION

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Abstract The effect of contralateral masking on a person's ability to detect small changes in frequency was studied. Eight normal hearing young adults with no previous participation in psychoacoustic experiments served as subjects. The task consisted of matching the frequency of a variable signal (3 575-4 425 Hz) to that of the reference tone (4 000 Hz) by the method of adjustment. As many tone pairs as necessary to complete the judgment were presented in a fixed temporal pattern. All subjects performed the task twenty-four times at both 20 and 40 dB SL with and without 40 dB SL of contralateral noise for a total of 96 judgments. The results indicated that contralateral broad band noise significantly improved the subjects' proficiency as a group to detect small changes in frequency although intersubject differences were noted. The presentation level of the reference and variable signals was shown to have little or no effect on scores.

The effects of contralateral masking on two site of lesion tests—Bekesy audiometry and the SISI—have been explored by Blegvad & Terkildsen (1966, 1967), Blegvad (1968, 1969), Tice (1968) and Osterhammel et al (1970). The results of those studies indicate that this form of masking can influence significantly the listener's differential sensitivity for changes in intensity.

Interest in monaural and binaural frequency discrimination as a promising diagnostic tool has been rekindled recently by Campbell (1970), Jones & Pracy (1971) and Carhart (1973). The Frequency Increment Sensitivity Test (FIST) developed by Campbell (1970) has been sug-

gested as another method which may be used to differentiate between normal and pathological ears. Based on a limited number of subjects, his data indicate that FIST is able to "differentiate between normal and damaged cochleae" (p. 376). In reviewing his results, Campbell reports that further investigations need to be conducted on several aspects of his procedure before it can be utilized routinely as a clinical tool. A recommendation of particular interest is that of studying the effect of contralateral masking on FIST scores. This concern is not limited to the FIST procedure alone but is a valid question with any measure of frequency discrimination.

The purpose of this study was to determine whether contralateral masking would influence the proficiency of normal hearing subjects in detecting small changes in frequency. Secondly, we wished to explore sensation level effects on frequency discrimination at 4 000 Hz with and without the presence of contralateral masking.

MATERIAL

Subjects

Eight young adults between the ages of 20 and 30 years (\bar{X} = 27.2) were selected based on the following criteria: (1) normal hearing

Table II Group means and standard deviations in Hertz of the difference between the reference and variable stimuli

Observation	Without contralateral noise Presentation level		With contralateral noise Presentation level	
	20 dB SL	40 dB SL	20 dB SL	40 dB SL
Mean	23.6	22.4	20.7	20.3
Standard deviation	21.1	19.2	18.8	19.6

rise and decay time of 50 msec was employed for both the reference and variable signals. An inter stimulus interval of 500 msec separated the reference signal from the variable signal. Successive pairs of tones were presented every 1.500 msec.

Test presentation order

All subjects were interviewed and received hearing evaluations prior to the experimental test session. Frequency discrimination measurements were obtained for 4000 Hz in the right ear under the following conditions: (1) at 20 and 40 dB SL without contralateral noise, and (2) at 20 and 40 dB SL with 40 dB SL of contralateral noise. Each subject made a total of ninety six judgments that is, twenty-four judgments for each of the four stimulus conditions. The sequence of each condition was varied systematically to preclude order and practice effects from biasing test results.

RESULTS

The data were analysed by means of analysis of variance employing a three factor approach for repeated measures (Winer, 1962). As indicated by the data in Table I, there was a significant difference between subjects in their ability to match the frequency of successive pairs of tones at 4000 Hz ($p=0.01$). Furthermore, frequency matching at 4000 Hz was not significantly affected by varying the sensation level at which the stimuli were presented. However, it was shown that the pres-

ence or absence of contralateral noise can significantly affect the size of the difference score ($p=0.05$). By looking at the mean scores and standard deviations for all subjects as a group in Table II, it can be seen that frequency matching differences were smaller for the conditions in which contralateral noise was applied to the non test ear. Also it should be noted that even though contralateral masking did improve the subject's ability to detect small changes in frequency as a group, this was not evidenced for each individual subject (Table III). Finally, there was no significant interaction exhibited between the variables.

As a point of interest, a corollary statistical analysis was performed on the number of paired stimuli required for the subjects to make a judgment under each of the conditions. Table IV indicates that neither presentation level nor contralateral masking significantly influenced the number of stimuli required to make a judgment ($p=0.05$). However, there was a significant difference between subjects regarding the actual number of stimuli required to make a judgment ($p=0.01$). The means and standard deviations for the number of stimuli presented under each condition are included in Table V.

In summary, it can be said that contralateral broad band noise did improve the subjects' proficiency, as a group, to detect small changes in frequency ($p=0.05$), while presentation level was shown to have little or no effect on their scores within the context of this design. Also it is clear that there were significant differences between subjects in their ability to match

Table III *Individual means and standard deviations in Hertz of the difference between the reference and variable stimuli*

Subject	Observation	Without contralateral noise Presentation level		With contralateral noise Presentation level	
		20 dB SL	40 dB SL	20 dB SL	40 dB S
1	Mean	23.7	23.6	27.7	22.3
	S.D.	15.0	20.9	21.0	16.7
2	Mean	13.0	11.1	12.5	10.0
	S.D.	7.4	7.2	9.4	8.8
3	Mean	45.3	34.3	34.9	41.0
	S.D.	33.7	20.8	25.3	28.0
4	Mean	9.7	15.2	11.7	15.4
	S.D.	8.5	13.6	8.6	13.7
5	Mean	20.8	23.1	19.3	17.0
	S.D.	14.6	16.5	16.1	16.4
6	Mean	25.0	21.2	13.8	17.8
	S.D.	16.4	13.6	12.5	14.6
7	Mean	31.9	36.1	29.2	21.0
	S.D.	23.2	24.9	20.8	17.0
8	Mean	19.1	14.8	16.1	17.7
	S.D.	13.8	13.8	13.8	19.7

the frequency of paired stimuli at 4000 Hz ($p=0.01$)

DISCUSSION AND CONCLUSIONS

Comparison with results of previous studies on DLFs in normal-hearing subjects

The difference limina for 4000 Hz obtained by Harris (1952), König (1957), and Parker et al (1968) can be compared to the results obtained in this investigation by studying the data presented in Table VI. Even though the subject's task and the psychophysical methods differ, the mean or median scores are quite similar. There is considerable divergency in data, however, between the standard deviations for the designs utilizing the method of adjustment and König's (1957) study which employed the method of constant stimuli. Whether this discrepancy is attributable to the differences in design or subjects, cannot be resolved within the limits of this study.

It is also interesting to note that König (1957) reports smaller DLF's as sensation level is increased from 10 to 40 dB. He failed to

mention whether these differences were statistically significant. Parker et al (1968) did not find statistically significant differences between scores obtained at 10 and 40 dB SL, nor did the present study find significant differences between scores obtained at 20 and 40 dB SL. Brandt (1963), in a detailed study of the DLF at 1000 Hz, found statistically significant improvements in frequency difference limina only as sensation level was increased from 0 to 5 dB. Above 5 dB SL, improvements in DLF as a consequence of increases in sensation level, were non-significant. The implications are that increases in sensation level of 10 dB or more do not produce smaller frequency difference limina, at least for the test paradigm used in this study and by Parker et al (1968).

Neural interaural interdependence

Numerous studies in audition have demonstrated the presence of neural interaural interdependence (Egan, 1948, Reynolds & Stevens, 1960, Prather, 1961, Vigran, 1965, Blegvad & Terkildsen, 1966, 1967, Blegvad, 1968, 1969, Tice, 1968, Osterhammel et al,

Table IV Summary of analysis of variance for all subjects as a group on the number of stimuli required to make a judgment utilizing two presentation levels, with and without the presence of contralateral masking

A = Subject
B = Presentation level
C = Noise level

Source of variance	Degrees of freedom	Sum of squares	Mean square	F
Between observations	191	18 726 801		
A	7	13 509 686	1 929 935	68 066*
Error A	184	5 217 115	28 354	
Within observations	576	12 388 750		
B	1	57 970	57 970	3 034
AB	7	190 207	27 172	1 422
Error B	184	3 515 073	19 104	
C	1	9 408	9 408	0 495
AC	7	156 603	22 372	1 178
Error C	184	3 494 239	18 990	
BC	1	4 230	4 230	0 166
ABC	7	275 155	39 308	1 543
Error BC	184	4 685 865	25 467	

* = significant at 0.01

1970 Rowley & Studebaker, 1971) Prather (1961) and Rowley & Studebaker (1971) support the contention that an increase in loudness with presence of contralateral noise is the result of central summation. These authors do not offer concrete evidence, however that would easily allow the researcher to accept their supposition as unequivocal. Vigran (1965) similarly reports that an increase in loudness can be associated with the presence of contralateral noise. He found that there was a greater increase in the loudness of a tone in the test ear when narrow bands of noise centered around the frequency of the test tone were used to mask the non test ear.

On the contrary Tice (1968) investigated the effects of contralateral narrow-band noise

on the SISI. He hypothesized that contralateral narrow-band noise, as compared to broad band noise, would increase the proficiency of listeners to detect small changes in intensity. He reasoned that an increase in the facilitation effect with contralateral narrow band noise would strengthen the notion that this increased proficiency was the sequela of binaural summation. Indeed, Tice did find an increase in the proficiency of listeners to detect small changes in intensity with the introduction of contralateral noise. There was not, however, a significant difference between SISI scores obtained with broad band and narrow-band contralateral noise. Consequently, Tice was not able to draw conclusions similar to those reported by Vigran (1965). Paul & Barry

Table V Group means and standard deviations for the number of paired stimuli that needed to be heard in order to make a judgment

Observation	Without contralateral noise Presentation level		With contralateral noise Presentation level	
	20 dB SL	40 dB SL	20 dB SL	40 dB SL
Mean	10.5	10.1	10.4	9.7
S.D.	7.0	6.3	6.5	5.6

Table VI *A comparison of pitch matching scores and DLFs for 4 000 Hz in normal hearing subjects*

Study	Mean	S D	Presentation level	No of subjects	Psychophysical method	Subject's task
Labiak (1971)	22.4 ^a	19.2	40 dB SL	8	Adjustment	Equal
Harris (1952)	21.0 ^b	Not given	30 dB SL	52	Constant stimuli	Higher or lower
Konig (1957)	21.2	6.8	40 dB SL	10	Constant stimuli	Higher or lower
Parker et al (1968)	18.5	19.3	40 dB SL	50	Adjustment	Equal

^a 40 dB SL without contralateral noise data only^b Median score

(1972) state that with the introduction of contralateral masking, detection of increments can be adversely affected. Based on their data, Paul & Barry speculate that the first few increments in a series may be less detectable due to an initial increase in neural activity level, but additional increments will be enhanced by a subsequent reduction in this activity. It would appear, then, that even though a facilitation effect has been shown to exist in the detection of small changes in frequency and intensity, the basis of the phenomenon cannot be defined clearly at the present time.

Clinical and research implications

Various studies (Meurman 1954, Butler & Albrite, 1956, Parker et al, 1968) have compared the frequency discrimination capacity of subjects with normal and pathologic ears. None of these studies employed contralateral masking to eliminate the possibility of cross conduction of the test stimulus. In fact, only one previous study (Campbell, 1970) has mentioned contralateral masking in relation to frequency discrimination scores. Thus it seems logical to conclude that any future clinical research conducted on frequency discrimination should consider the possible influence of contralateral masking. In addition, since the design of this study was limited to one level

of contralateral noise, one frequency, and a limited population, future investigations should explore these parameters in greater detail.

It is also recommended that caution be exercised regarding the choice of psychoacoustic methodology to be employed while evaluating frequency discrimination. Harris (1952), Konig (1957), and Parker et al (1968) contend that the frequency modulation technique, often used in clinical situations, is not a valid measure of frequency discrimination due to the inherent artifacts introduced with this procedure. It is suggested that the method of constant stimulus or method of adjustment in combination with successively paired tone bursts be adapted for clinical investigations.

Although there is still a paucity of data regarding the effects of various pathologies on frequency discrimination, our knowledge is increasing. This study and previous investigations have suggested possible test paradigms and associated precautions for evaluating these phenomena. Hopefully, an in depth resurgence in the study of this aspect of audition will prove to be clinically rewarding.

ACKNOWLEDGMENT

The authors express their appreciation to Dr Wendel Walton for his assistance in instrumenting this study.

Table IV Summary of analysis of variance for all subjects as a group on the number of stimuli required to make a judgment utilizing two presentation levels, with and without the presence of contralateral masking

A = Subject
 II = Presentation level
 C = Noise level

Source of variance	Degrees of freedom	Sum of squares	Mean square	F
Between observations	191	18 726 801		
A	7	13 509 686	1 929 955	50.066*
Error A	184	5 217 115	28 354	
Within observations	576	12 388 750		
II	1	57 970	57 970	3.034
AB	7	190 207	27 172	1.422
Error II	184	3 515 073	19 104	
C	1	9 408	9 408	0.495
AC	7	156 603	22 372	1.178
Error C	184	3 494 239	18 990	
BC	1	4 230	4 230	0.166
ABC	7	275 155	39 308	1.543
Error BC	184	4 685 865	25 467	

* = significant at 0.01

1970 Rowley & Studebaker, 1971) Prather (1961) and Rowley & Studebaker (1971) support the contention that an increase in loudness with presence of contralateral noise is the result of central summation. These authors do not offer concrete evidence, however, that would easily allow the researcher to accept their supposition as unequivocal. Vigran (1965) similarly reports that an increase in loudness can be associated with the presence of contralateral noise. He found that there was a greater increase in the loudness of a tone in the test ear when narrow bands of noise, centered around the frequency of the test tone, were used to mask the non test ear.

On the contrary, Tice (1968) investigated the effects of contralateral narrow-band noise

on the SISI. He hypothesized that contralateral narrow band noise, as compared to broad band noise, would increase the proficiency of listeners to detect small changes in intensity. He reasoned that an increase in the facilitation effect with contralateral narrow band noise would strengthen the notion that this increased proficiency was the sequela of binaural summation. Indeed, Tice did find an increase in the proficiency of listeners to detect small changes in intensity with the introduction of contralateral noise. There was not, however, a significant difference between SISI scores obtained with broad band and narrow band contralateral noise. Consequently, Tice was not able to draw conclusions similar to those reported by Vigran (1965). Paul & Barry

Table V Group means and standard deviations for the number of paired stimuli that needed to be heard in order to make a judgment

Observation	Without contralateral noise Presentation level		With contralateral noise Presentation level	
	20 dB SL	40 dB SL	20 dB SL	40 dB SL
Mean	10.5	10.1	10.4	9.7
S.D.	7.0	6.3	6.5	5.6

SUBMIKROSKOPISCHE VERÄNDERUNGEN DER REISSNERSCHEN MEMBRAN NACH STREPTOMYCINAPPLIKATION

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Abstrakt Wir beobachteten in den Epithelial-, aber auch Mesenthelialzellen eine große Aktivität und neben der Beeinträchtigung der normalen Aktivität die Zerstörung der Zellen von mesenthelialer Seite her. Es läßt sich sagen, daß die RM entsprechend Versuchsdauer und angewandtem Antibiotikum sowie Dosierung nach einem Gradienten von der Peri- zur Endolymphe hin zerstört wird, da die Panozytose von der Basalmembran ihren Ausgang nimmt und zu einer deutlichen Vakuolisierung der Mesenthelialzellen führt. Desgleichen sind die Veränderungen im Bereich der Zonula occludens und des Desmosomen von Bedeutung.

Die Reissnersche Membran (RM) ist für das Innenohr von besonderer Bedeutung, da sie als biologische Membran sowohl aktiv als auch passiv die Austauschvorgänge zwischen Peri- und Endolymphe beeinflusst.

Submikroskopische Untersuchungen der normalen Struktur der RM wurden wiederholt durchgeführt (Robertson, 1959, Hagisawa, 1963, Iurato, 1967, Duvall & Sutherland, 1970, Iurato & Tardelli, 1971). Sie besteht aus zwei unterschiedlichen Zellagen, die durch eine Basalmembran voneinander getrennt sind. Die Bindegewebszellen auf der Seite der Scala vestibuli sind zart, Mikrovilli fehlen. Die der Scala media zugewandte epitheliale Zellage ist durch sogenannte Mikrozytosevesikel, einen gut entwickelten Golgiapparat im Zytoplasma und zahlreiche Mikro-

villi zur Endolymphe hin gekennzeichnet. Watanuki beobachtete, daß die Epithelialzellen zum Limbus spiralis hin schmaler sind als die zum Ligamentum spirale.

Die Pathologie und Histochemie der Schädigung des Innenohres durch Streptomycinpräparate wurden, wie die Untersuchungen von Peri- und Endolymphe, wiederholt beschrieben (Kohonen, 1965, Musebeck & Schatzle, 1962, etc.). Wie man glaubt, sind die Wirkungen des Streptomycins auf die lebende Zelle folgende:

1. Beeinträchtigung der Funktion der Zellmembran und

2. Hemmung der Proteinsynthese der Zelle.

Die primäre Schädigung von Dihydrostreptomycin (DHS) lokalisiert sich in den Mitochondrien (Duvall & Wersall, 1964, Musebeck, 1963) oder in den Ribosomen (Spoendlin, 1966) und entspricht im wesentlichen den von Brock (1966) gefundenen Einwirkungen von Antibiotika auf Bakterien.

Die angeführten Änderungen der normalen Strukturen erscheinen als Resultat der Einführung der Antibiotika in die Endolymphe über die Stria vascularis und die RM.

Submikroskopischen Untersuchungen der Reissnerschen Membran nach Streptomycinapplikation kommt eine besondere Bedeutung zu. In der Literatur fehlen diese Untersuchungen (Spoendlin et al., 1971).

Herrn Prof. Dr. sc. med. Kurt Dietzel zum 60. Geburtstag gewidmet.



Abb 1 RM nach der im. Injektion von 10×250 mg/kg Körpergewicht Streptomycinsulfat. In den epithelialen Zellen finden sich fingerartige Fortsätze, die sich zur Seite der verdickten Basalmembran hin erstrecken. Beginnende Vergrößerung des Zwischenzellspaltes im Bereich des Desmosomen, der Zona occludens. Beginnende Vakuolisierung und Vergrößerung der Lysosomen der mesenchymalen Zellen 9 100 \times

MATERIAL UND METHODE

Zwanzig ohrgesunde Meerschweinchen, etwa 250 g schwer, erhielten nach der Prüfung mittels Preyerschem Ohrmuschelreflex 250 mg Streptomycinsulfat/kg Körpergewicht/die. Nach 5, 10, 15 und 20 Tagen wurden je 5 Tiere der Versuchsserie entnommen und beide Cochleae nach der Methode von Vinnikov & Titova (1964) gewonnen. Alle Cochleae wurden nach sofortiger Fixierung in 1%iger OsO₄ und der Aufarbeitung nach der Methode von Kolfield in Epon eingebettet, die Ultradünnschnitte mit dem Ultratome III (LKB Stockholm) angefertigt und mit dem Elektronenmikroskop JEM 6c untersucht.

UNTERSUCHUNGSERGEBNISSE

Die ersten auffälligen Veränderungen an der RM wurden nach 10 Injektionen beobachtet (Abb 1). In den epithelialen Zellen kommen fingerartige Fortsätze zur Beobachtung, welche sich zur Seite der Basalmembran hin erstrecken. Außerdem sind Verdickungen der Basalmembran zu beobachten. Auf der Seite der Basalmembran sieht man eine verstärkte Neubildung von Lysosomen. An den Kontaktstellen zweier Zellen in dem Gebiet des Desmosomen, der Zona occludens kommen deutliche Vergrößerungen des Zwischenzell-

spaltes zur Beobachtung. Die der Scala vestibuli zugewandte Seite der Zelle befindet sich jedoch in einem relativen Zustand der Ruhe. In den mesenchymalen Zellen beobachtet man eine Vergrößerung der Lysosomen und eine beginnende Vakuolisierung. Zwischen benachbarten Zellen sieht man größere Lücken mit Flüssigkeit. Im Bereich dieser findet sich eine Hyperplasie des Desmosomen. An der vestibulären Seite der Epithelialzelle nimmt anscheinend vorübergehend die Zahl der Mikrovilli zu. Im Zytoplasma werden große Lipidgranula beobachtet (Abb 2). Sehr demonstrativ sind die Veränderungen nach dem 15. Tag. In diesem Stadium beobachtet man eine Zunahme der Flüssigkeit zwischen den Epithelialzellen. Im Bereich zwischen Epithelial- und Mesenchymalzelle kommen vereinzelt Ausfälle von Niederschlägen zur Beobachtung. An der Stelle, an welcher die Epithelialzellen miteinander Kontakt haben, vergrößert sich der Durchmesser des Zwischenzellspaltes, aber die Desmosomen und die Zona occludens bleiben sehr deutlich. Sehr deutlich sind die Erscheinungen von Vakuolisierung und Pinozytose, Mikrozytosevesikel vermindern sich (20. Versuchstag, Abb 3).

In den Mesenchymalzellen nehmen Zahl und Größe der Lysosomen zu. Außerdem sieht man auch in diesen Zellen eine deutliche Vakuolenbildung.

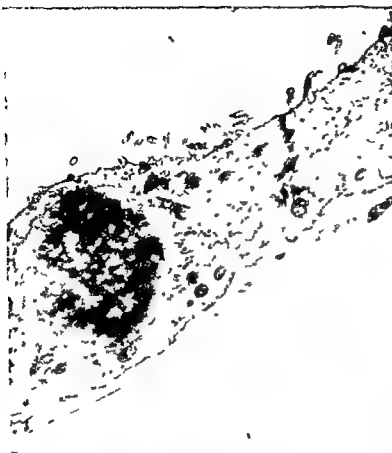


Abb 2 RM nach der im Injektion von 15×250 mg/kg Körpergewicht Streptomycinsulfat. Es zeigen sich starke Lipidanhäufungen im Zytoplasma der Epithelzelle und eine Hyperplasie des Desmosomen 13 800 \times

Nach dieser Versuchsdauer sind Verschmelzungen der Basalmembran, Polysomenanhäufungen (Abb 4), Vermehrung von freien Ribosomen (Abb 5) sowie eine weitere Abnahme von Mikrozytosevesikeln nachweisbar und eine Vergrößerung der Lysosomen besonders auf der den mesenchymalen Zellen zugewandten Seite (Abb 6). Die Zellorganellen werden zunehmend elektronenoptisch dunkler.

Alle diese beobachteten Faktoren deuten darauf hin, daß — unter den gegebenen Versuchsbedingungen — zu einer starken Veränderung der Permeabilität von der Peri zur Endolympe hin kommt. Wir glauben, daß es nach der Gabe von Antibiotika primär zu Permeabilitätsstörungen durch Veränderungen in den Zellmembranen kommt, während die pathologischen Veränderungen in der Zellen sekundärer Natur sind.

DISKUSSION

Das Streptomycin beeinträchtigt die Permeabilität der RM stark. Die pathologischen Veränderungen, die diese Beeinträchtigung hervorrufen haben einen progredienten Faktor, der sich offensichtlich von der Peri zur Endolympe hin ausbreitet. Diese Schlußfolgerungen ergeben sich aus den vorliegenden Ergebnissen.

Beobachtet werden eine ausgeprägte Pinozytose, eine teilweise Verschmelzung der Basalmembran zwischen Epithelial und Mesothelialzellen, Luckenbildung im Bereich der Basalmembran. Die Zwischenzellspalten vergrößern ihren Durchmesser, füllen sich mit Flüssigkeit an, während die Desmosomen und die Zonula occludens erhalten bleiben. Auf Grund unserer Untersuchungen möchten wir entsprechend Duvall et al (1969) and Duvall



Abb. 3 RM nach der im Injektion von 20×250 mg/kg Körpergewicht Streptomycinsulfat. Zwischen den Mesothelial und Epithelialzellen werden Aus-

flockungen beobachtet, weiter starke Lipidanhäufungen sowie eine Vakuolisierung und Pinozytose im Zytoplasma der Epithelialzellen. $21000 \times$

& Sutherland (1970) zwei potentielle Möglichkeiten des Durchdringens der RM von der Peri- zur Endolympe annehmen: 1. durch die Pinozytose und 2. über die Zwischenzellspalten. Eine zunehmende Vakuolisierung sowie eine Verminderung von Mikrozytoseblaschen und zunehmend elektronenoptisch dunkler werdenden Zellorganellen erinnern an die von Kaneko et al. (1970) und Hawkins (1970) erhobenen Befunde. Eine unterschiedlich starke elektronenoptische Dichte des Zytoplasmas, wie sie Kaneko et al. (1970) nach der Gabe von Kanamycin, welche zur Differenzierung der RM in dunkle und helle Zellen führte, sahen, konnte von uns, ähnlich wie Hawkins (1972), nicht beobachtet werden. Ciges et al. (1972) sahen ein verändertes Aufnahmeverhalten von Neutralrot der RM nach Kanamycinintoxikation.

Hervorgerufen werden diese pathologischen Prozesse durch eine Zunahme der Konzentration des Streptomycins primär in der Perilymphe, wodurch es schließlich zu einer Zer-

störung der membranösen Strukturen der Zellen kommt.

In Zusammenhang mit der fortschreitenden Zerstörung der membranösen Struktur der Zellen, dem Verschwinden der SDH nach Streptomycingabe und dem Schutzmechanismus des Cytochrom C muß man auch eine Beeinträchtigung des Sauerstoffmetabolismus bei Streptomycinintoxikation als gesichert ansehen.

Durch die steigende Konzentration des Präparates in der Perilymphe wird die RM geschädigt, wodurch ihre Funktion als effektive biologische Trennmembran zwischen Peri- und Endolympe beeinträchtigt wird.

Die zwischen den Epithelial und Mesothelialzellen beobachteten Niederschläge konnten durch eiweißartige Flockungen oder — wie Schatzle (1972) meint — durch das Streptomycin ausgefallene saure Mucosaccharide bedingt sein.

Nach Kanamycinbehandlung, Hypoxie und Lärmbelastung kommt es zu einer Permeabi-

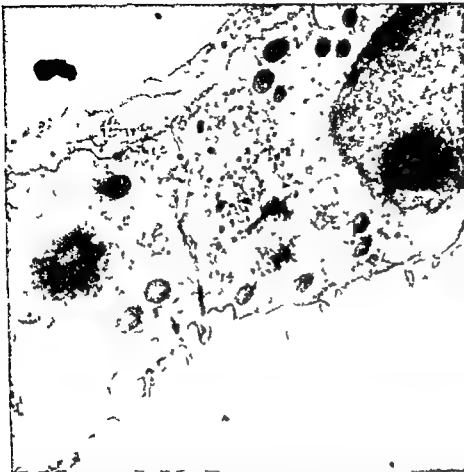


Abb 4 RM ebenfalls nach 20 Injektionen (s o) Besonders deutliche Verminderung der Mikrozytosevesikel des Zytoplasmas Ausflokkungen zwischen beiden

Zellagen sowie ein auffallend breiter Zwischenzellspalt benachbarter Epithelzellen und Polysomenanhäufungen sind nachzuweisen 23 000 \times

litatssteigerung der RM (Misrahy et al, 1962) Von Stupp (1971) wird unter ähnlichen Versuchsbedingungen zwar eine deutliche Zunahme der Kaliumkonzentration in der Perilymphe bestätigt als Ursache aber nicht unbedingt eine Verminderung des aktiven Kationentransportes durch die RM diskutiert Möglich wäre auch eine scheinbare Hemmung des Kaliumdurchtrittes durch die RM als Folge einer verminderten Resorptionsfähigkeit der Stria vascularis, wodurch zwangsläufig der Flüssigkeitsstrom und Ionen transport von der Scala tympani durch die RM zum Endolymphraum hin zum Erliegen kommen konnte

Die histochemischen Untersuchungen des

Innenohres auf Proteinbausteine von Musebeck & Schatzle (1962) ergaben nach den Methoden von Danielli und Burstone eine mittelgradige, positive Reaktion der RM Die respiratorische Rate der RM verglichen mit anderen Geweben ist außerordentlich hoch

Der Eiweißstoffwechsel der RM gemessen durch die Bestimmung der Eiweißbaurate beträgt im Gegensatz zur hohen respiratorischen Rate nur 50% des Eiweißstoffwechsels der Ganglienzellen und liegt auch unter dem der Stria vascularis Watanuki et al (1968) untersuchten den RNS-Stoffwechsel der Zellen der RM und schlußfolgern auf Grund unterschiedlicher Ergebnisse für die inneren mediolusnahen und die äußeren



Abb 5 RM ebenfalls nach 20 Injektionen (s.o.) Freie Ribosomen treten vermehrt auf Zunahme von Lysosomen in den Mesenchymal aber auch Epithelialzellen deutliche Vakuolisierung des Zytoplasmas 7500 1

Zellen der RM eine unterschiedliche Funktion der Permeabilität

Nach der Meinung von Duvall & Wersall (1964) und Musebeck (1963) lokalisiert sich der primäre Streptomycineffekt von DHS in den Mitochondrien oder in den Ribosomen (Spoendlin 1966). Da es nach der Streptomycinapplikation in Abhängigkeit von der Dosis und der Anzahl der Injektionen zu einer submikroskopisch nachweisbaren Zerstörung aller membranösen Strukturen der Zellen kommt, sind die angeführten Möglichkeiten der Ansatzpunkte der Schädigung im Zellstoffwechsel außerordentlich bedeutungsvoll.

Die Depression der nuklearen RNS-Synthese und die Abnahme des zytoplasmatischen RNS-Gehaltes im Innenohr nach Streptomycinapplikation läßt vermuten, daß schon bei relativ niedrigen Dosen zytoplasmatische Ribosomen und chromosomaler Apparat betroffen sind. Diese vermutete Beeinträchtigung läßt sich auch für die Zellorganellen der RM nach Streptomycinapplikation nachweisen.

Bei den in der RM an Normaltier histochemisch nachgewiesenen Lipiden handelte es sich durchweg um sogenannte maskierte Fette. Mit der Benzpyren-Fluoreszenznachweis-

methode ergab sich für die RM eine positive Reaktion (Schatzle & Westernhagen, 1967), wie sie auch mit der Sudanschwarz bzw. Sudanrot-Methode mitgeteilt wurde. Diese Befunde können jedoch nicht die von uns gefundenen Lipidtropfen erklären. Schätzle (1972) vermutet, daß es sich hierbei um ein durch Streptomycinschädigung hervorgerufenes sekundäres Phänomen durch Ansammlung lysosomaler Lipide handelt. Von Hawkins (1972) und Ross (1972) wurde vermutet, daß es sich hierbei um Lysosomen handle, da sich bei der Reaktion auf Na^+ offensichtlich eine dieses Gebilde umgebende Membran darstellt. Koitschev fand in dieser jedoch keine ATPase.



Abb 6 RM ebenfalls nach 20 Injektionen (s.o.) Elektronenoptisch dunkles großes Lysosom auf der mesothelialen Seite einer Epithelialzelle 28 000 1

Herrn Prof. Dr. J. A. Vinnikov und Frau Prof. Dr. L. E. Titova möchte ich für die Unterstützung und Förderung bei der Durchführung dieser Arbeit meinen herzlichen Dank sagen.

SUMMARY

Intense activity was observed in both epithelial and in mesothelial cells, and, in addition to disturbance of normal activity, destruction of cells from the mesothelial wall could also be detected. It remains to be seen whether, according to the duration of test and the antibiotic used, as well as the dosage, Reissner's membrane is destroyed along a gradient from the peri to the endolymph side, since the pinocytosis derives from the basal membrane and leads to an evident vacuolization of the mesothelial cells. In the same way, the changes within the zona occludens and in the desmosomes are also of importance.

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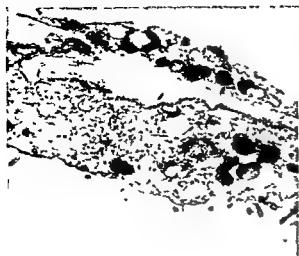


Abb 5 RM ebenfalls nach 20 Injektionen (s.o.) Freie Ribosomen treten vermehrt auf, Zunahme von Lysosomen in den Mesenchymal aber auch Epithelialzellen deutliche Vakuolisierung des Zytoplasmas 7 500 \times

Zellen der RM eine unterschiedliche Funktion der Permeabilität

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Bei den in der RM an Normaltier histochemisch nachgewiesenen Lipiden handelte es sich durchweg um sogenannte maskierte Fette. Mit der Benzpyren-Fluoreszenznachweis-

methode ergab sich für die RM eine positive Reaktion (Schatzle & Westernhagen, 1967) wie sie auch mit der Sudanschwarz- bzw. Sudanrot-Methode mitgeteilt wurde. Diese Befunde können jedoch nicht die von uns gefundenen Lipidtropfen erklären. Schatzle (1972) vermutet, daß es sich hierbei um ein durch Streptomycinschädigung hervorgerufenes sekundäres Phänomen durch Ansammlung lysosomaler Lipide handelt. Von Hawkins (1972) und Ross (1972) wurde vermutet, daß es sich hierbei um Lysosomen handle, da sich bei der Reaktion auf Na^+ offensichtlich eine dieses Gebilde umgebende Membran darstellt. Koitschev fand in dieser jedoch keine ATPase.



Abb 6 RM ebenfalls nach 20 Injektionen (s.o.) Elektronenoptisch dunkles, großes Lysosom auf der mesothelialen Seite einer Epithelialzelle 28 000 \times

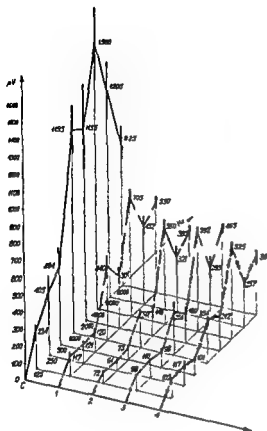


Fig. 1 Curve of CM values after 12 weeks in μV in the various groups of animals C Control group I Group exposed to noise 2 Group given kanamycin for 1 week before 12 weeks exposure to noise 3 2 weeks kanamycin before 12 weeks exposure to noise 4 2 weeks kanamycin during 12 weeks exposure to noise Acoustic stimulus = 80 dB

The Preyer reflex was tested in all the animals before the experiments were begun and animals with a weak reflex were not included in the experiments

RESULTS

The mean values of the CM and AP voltages were determined the standard deviations calculated and a statistical analysis of the results was made on the basis of the unilateral Dunnett test. The mean from the control group was taken as the standard in the Dunnett test and the results obtained from the other groups

were analysed in relation to this standard $\alpha = 0.05$ was taken to be the significance level. The calculations were made on a computer Odra 1204 at the Electronic Computation Technique Centre of the Institute of Energetics Warsaw. Most of the findings were statistically significant.

In all the groups of animals the changes in CM voltage were found to be phasic. In group I the animals exposed to noise only the greatest percentage fall in CM voltage was observed 1 week after this exposure and a similarly large fall in CM continued up to 2 weeks of exposure to noise. This fall was noted at all frequencies but the greatest occurred at 2000 Hz (77%) and 6000 Hz (84%). After the 3rd and 4th week a gradual fall in CM was observed in the low and medium frequency ranges whereas the CM voltage at high frequencies (from 4000–8000 Hz) rose. A period of 6–12 weeks exposure to noise caused a gradual rise in CM at all frequencies. The CM voltage after 12 weeks exposure exceeded that of the CM voltage noted after 2 weeks. When an acoustic stimulus of 80 dB was applied the greatest fall in CM was observed at frequencies of 2000 Hz (74%) and 500 Hz (76%). With a stimulus of 60 dB however the fall in CM occurred at 2000 Hz (77%) and at high frequencies 6000 Hz (74%) and 8000 Hz (71%).

The dynamics of the CM changes in animal group II to which kanamycin was administered 1 week before exposure to noise were similar the only difference being that after 12 weeks exposure a marked fall in CM occurred at all frequencies the greatest percentage fall occurred at 2000 Hz (87%) with an acoustic stimulus of 80 dB and when 60 dB were applied the fall occurred at high frequencies 6000 Hz (91%), 8000 (88%) and at a frequency of 2000 Hz (89%).

In group III which received kanamycin 2 weeks before exposure to noise the first phase of the fall in CM was prolonged continuing up to the end of the 3rd week. After 4 weeks a gradual rise in CM was observed beginning

with the high frequencies and, after 6 weeks, occurring at all frequencies. After 12 weeks, however, a marked fall in CM at all frequencies was noted. The greatest percentage fall occurred with an 80 dB stimulus at 2000 Hz (84%) and with an intensity of 60 dB this fall was noted at the high frequencies, 6000 Hz (88%), 8000 Hz (82%), and at 2000 Hz (85%).

The first phase of the CM fall continued for 3 weeks in the group IV which received kanamycin for 2 weeks while being exposed to noise. The greatest percentage fall occurred when both these factors, noise and kanamycin, affected the hearing simultaneously. The CM rose at all frequencies after 4 weeks, but after 11 weeks, i.e. earlier than in the previous groups, another depression of CM occurred which deepened in the 12th week. When tested with tones of 80 dB, the greatest percentage fall was noted at 2000 Hz

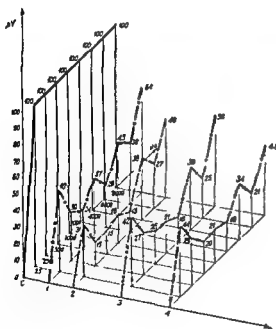


Fig 2 Curve of CM values per cent after 12 weeks in the various groups of animals C Control group I Group exposed to noise 2 Group given kanamycin for 1 week before 12 weeks exposure to noise 3 2 weeks kanamycin before 12 weeks exposure to noise 4 11 weeks kanamycin during 12 weeks exposure to noise Acoustic stimulus = 80 dB

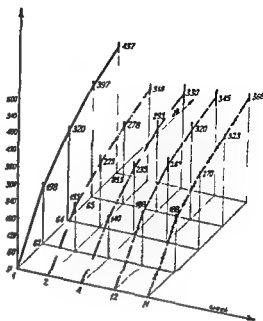


Fig 3 Curve of AP values in μV in group I in comparison with control group N Control group After exposure to noise for 1 week (1) 2 weeks (2) 4 weeks (4) 12 weeks (12)

(81%) and with tones of 60 dB at 6000 Hz (84%), 8000 Hz (84%) and 2000 Hz (81%).

Together with these tests, an analysis of the AP values noted in the various groups was carried out. The analysis was based on comparison of the N_1 AP amplitude after 1, 2, 4, and 12 weeks. In groups I, II, and III, the changes in AP voltage were analogous. After 1 week's exposure to noise, the AP voltages rose to a level higher than that of the control group. After 2 weeks, the AP fell below that of the control group. After 4 weeks there was no tendency to a fall in AP. After 12 weeks, the AP values equalled those of the controls in group I, whereas the AP values of group II and III were slightly higher than the control values.

The dynamics of the AP changes in group IV, animals which were given kanamycin while being exposed to noise, differed somewhat. The first phase, during which the AP values exceeded those of the controls lasted for a whole 2 weeks and the second phase

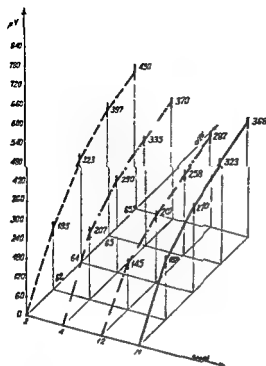


Fig. 4. Curve of AP values in μV in group IV N Control group. After exposure to noise for 2 weeks (2) 4 weeks (4) 12 weeks (12).

that of a fall in AP values, lasted to the end of the 12th week.

The peripheral adaptation was also studied by comparing the values of unadapted N_1 AP by increasing the frequency of the stimulus (100, 200, 400, 500 clicks/sec).

In all the groups of animals, their adaptive capacity was retained, its increase being a function of the stimulus frequency. In all the experimental groups, the adaptation was less than that of the controls. The adaptation of the controls was found to be 22%–34%, that of group I, 10%–15%, of group II, 12%–17%, of group III, 13%–19%, of group IV, 15%–21%. No marked regularity of adaptation in relation to the period of exposure to noise was noted (the results were not statistically significant). There was only a greater variation in adaptation in the groups of animals subjected to both noise and kanamycin.

DISCUSSION

Most authors have observed a fall in CM and AP when the animal is exposed to noise (Gisselsson & Sørensen, 1959, Lawrence et al., 1959, Ruedi & Furrer, 1947, Sekula & Trajka, 1967) but the dynamics of the CM and AP changes over a longer period of time have not so far been published. Jankowski et al. (1971) observed the dynamics of CM and AP changes over a period of 1–6 days.

As has been reported by other authors (Hawkins, 1959, Jankowski et al., 1971, Lawrence et al., 1959) and as was found in our investigations, the fall in CM occurs at all frequencies. In our investigations the extent of the fall in CM voltage was greater when an acoustic stimulus of a lower intensity, 60 dB, was applied (this is in agreement with the findings of Wagner & Gerhardt, 1963) and it was noted that the fall in CM occurs above all at the high frequencies (similar results were reported by Jankowski et al., 1971) and at a frequency of 2000 Hz, as was observed by Lawrence et al. (1959).

The dynamics of the changes in CM had a marked phasic character regardless of the intensity of the acoustic stimuli. The first phase, lasting up to 2 weeks inclusive, was characterized by the greatest percentage fall. During the second phase, which lasted 4 weeks, a certain stabilization occurred, characterized by a minimal fall in CM at low and medium frequencies and a rise in CM at high frequencies. The third phase, of 12 weeks' duration, brought a further restitution of CM at all frequencies. In the animals which received kanamycin before being exposed to noise and while being exposed to noise, a fourth phase was observed, that of another fall in PM voltage. This phase occurs earlier (after 6 weeks' exposure) in the animals which received kanamycin while being exposed to noise than in those animals which were given kanamycin before exposure (after 12 weeks).

On the basis of extensive comparative studies, Wever (1966) found that there is a marked

In all the groups of animals, the peripheral adaptation which was preserved, though lower than that of the control group, is, according to Rosenbluth (1954) and Stange et al (1964) evidence of deterioration in the function of the transformation organ

CONCLUSIONS

- 1 The dynamics of the electrocochleogram changes (CM and AP) in the guinea pig while under the influence of industrial noise, and kanamycin + industrial noise are phasic
- 2 The phase of the changes in CM and AP differs
- 3 The phases of the changes in CM in the animals subjected to industrial noise for 12 weeks consisted of a phase of fall, a phase of stabilization and a phase of regression. When the animals were exposed to two harmful agents another phase of a secondary fall occurred
- 4 The changes in AP were characterized by periods of rise, fall, and regression, in the animals which were exposed to both noise and kanamycin at the same time, the phases of rise and fall were prolonged
- 5 The extent of the fall in CM depends on the intensity of the acoustic stimulus used for the investigations
- 6 In the regression phase, the rise in CM voltages begins at the high frequencies
- 7 Peripheral adaptation to industrial noise and kanamycin deteriorates
- 8 The findings confirmed by statistical methods indicate that kanamycin predisposes the ear to damage by industrial noise, particularly when this drug is administered during exposure to such noise

ZUSAMMENFASSUNG

Bei 250 während einer bis zu 12 Wochen mit Industrielärm sowie Kanamycin und Lärm belasteten Meerschweinchen wurde die Veränderungsdynamik des Elektrocochleogramms (CM, AP) untersucht. Es wurde eine Phasendynamik der CM- und AP-Veränderungen sowie eine bewährte aber herabgesetzte periphere Adaptation festgestellt. Die erzielten statistisch be-

stätigten Ergebnisse weisen auf einen prädisponierenden Einfluss des Medikaments mit ototoxischer Nebenwirkung auf die Beschleunigung der industriellen Beschädigung des Gehörs hin und auf eine besonders schnelle Wirkung bei Verabreichung des Mittels während der Lärmbelastung.

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TEMPORARY HEARING LOSSES IN TEEN-AGERS ATTENDING REPEATED ROCK-AND ROLL SESSIONS

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Abstract This study investigated the hearing of teen-agers who voluntarily exposed themselves to repeated sessions of loudly amplified pop music. Hearing thresholds were measured before and 30 min after exposure for each of 8 weekly sessions of rock-and roll music with an average sound pressure level of 110 dB to 115 dB. Significant temporary threshold shifts were found in all subjects, especially in the high frequencies. Hearing sensitivity recovered between the repeated exposures, and a 5 month follow up study found that the hearing in all subjects but one had returned to its initial pre-exposure level. The exposure had differential effects on the two ears at the same test frequencies.

Increasing concern about environmental noise pollution has prompted studies other than those in which acoustic trauma has been experimentally induced or attributed to industrial or military exposures to loud sounds. In the past decade the majority of our young people have voluntarily exposed themselves to highly amplified rock and roll, discothèque, or pop music. This type of music has been investigated

from several different points of view. Measurement of sound pressure levels and frequency distributions led Lebo & Oliphant (1968) to conclude that prolonged exposure to such music was unsafe. Kryter (1970) described rock and roll as intermediate between impulsive and non impulsive sound and pointed out that it exceeded limits set forth in damage risk criteria. Rintelmann & Borus (1968) investigated musicians who were exposed to this type of music as an occupational hazard and reported that only 5% of 42 performers had permanent hearing losses after long term exposure. Most workers have agreed that rock and roll produced at least temporary changes in the threshold of hearing. Rice et al (1968), Speaks et al (1970) and Jerger & Jerger (1970) measured shifts in the hearing thresholds of performers at 2 min, 20 to 40 min, and 1 hour respectively after exposure. Temporary hearing losses were present in almost all subjects, and permanent losses were recorded in about 25% of the musicians. Laboratory studies because of the recognized risk to hearing have necessarily limited the exposure of subjects to rock and roll music to one session of an hour or less (Dey, 1970, Hickling 1970). Recently Rintelmann et al (1972) had 20 subjects listen to an hour of rock and roll music under both continuous and intermittent conditions. The hearing recovery pattern was fol-

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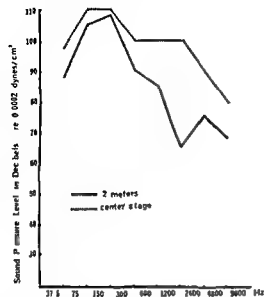


Fig 1 Average octave band frequency spectrum. Data points represent the average measurement for the bandwidth between the two frequencies indicated at either side of each data point.

lowed up to 90 min after exposure, and it was concluded that daily exposures over an extended period would be hazardous to hearing.

The purpose of the present study was to investigate the temporary and any possible permanent noise induced hearing losses in a group of teen agers actually exposed to long hours of highly amplified live rock and roll music over a series of eight weekly discotheque sessions. These sessions were part of a city summer recreational program for young people and were held in an outdoor amphitheater. Each session was frequented by well over a thousand teen agers. Exposure of human subjects to such loud sound for several hours over repeated sessions is not readily possible in a laboratory setting.

Of the randomly selected teen agers who were willing to have their hearing tested before and after each weekly rock and roll session, 14 completed the project. These included 4 boys and 10 girls ranging in age from 13 to 17 years. None had any history of ear disease, familial hearing loss, or ingestion of ototoxic drugs.

Two calibrated audiometers were set up in separate rooms of a quiet basement in a home a short distance from the park where the rock and-roll sessions took place. Ambient noise level, measured in the test environment for each session, did not exceed 40 dB sound pressure level (SPL). Two experimenters administered pure tone hearing tests to the subjects before each rock-and-roll session and again 30 min after the session. The same tester and the same audiometer were used on each occasion for a given subject. Both ears were tested by air conduction at 250, 500, 1000, 2000, 4000, and 8000 Hertz (Hz) for both pre-exposure and post-exposure measurements for the eight weekly sessions. Pure tone bone conduction thresholds were measured at the initial pre exposure test and at the follow up hearing evaluation conducted 5 months after the last rock-and-roll program.

The sound pressure level and frequency distribution of the rock-and-roll music were measured weekly using the slow setting and both A and C filters of a Rudmose RA-100 Sound Level Analyzer. Measurements were taken at different positions around the large stage. Fig 1 displays an average of the sound level meter readings for the various frequency bands at 2 meters in front of the performers and at center stage. At the farthest edge of the stage the SPLs were about 10 dB less. At a distance of the sixth row in the amphitheater, the overall SPL was 90 dB to 95 dB. Side and center stage readings had an overall average of 110 dB and 115 dB SPL respectively. The loudest range of the spectral distribution was from 75 Hz to 1200 Hz with a slight peak between 300 Hz and 600 Hz. There were times during measurements of the sound levels that the SPL peaked beyond the 150 dB maximum capacity of the meter. The rock and roll music was intermittent in that there was about a minute between selections. When one group of performers took a rest, a second combo played after an interval of less than 5 minutes. Each weekly session lasted for 3 1/2 hours.

Table I Average temporary threshold shift in dB for eight rock and roll sessions

	250 Hz	500 Hz	1 000 Hz	2 000 Hz	4 000 Hz	8 000 Hz
<i>Right ear</i>						
79		43	18	107	182	164
<i>Left ear</i>						
11		00	71	139	154	111

RESULTS

The 30 min post exposure audiograms showed temporary threshold shifts (TTS) for all subjects at three or more of the six test frequencies in both ears. Table I displays the average threshold shift found for each ear at each frequency. Individual subjects varied widely in the amounts of TTS in their post exposure audiograms, but all subjects demonstrated the most TTS at 4 000 Hz with 2 000 Hz and 8 000 Hz affected to a lesser degree.

When pure tone test results for before and after the rock-and-roll sessions were compared, the *t* scores were statistically significant at the 95% level of confidence for 2 000, 4 000, and 8 000 Hz in both ears. Therefore, even after 30 min of recovery time, the amount of temporary hearing loss present at these frequencies was significant.

Pure tone hearing thresholds for each subject were highly correlated across pre-exposure measurements, indicating good intra subject reliability. Each pre exposure audiogram showed that the subject's hearing sensitivity had recovered from the TTS of the previous week's exposure. Subjects who initially had small threshold shifts tended to have less TTS for succeeding rock and roll sessions than subjects who initially had large shifts in hearing levels. When post exposure thresholds for the first and last sessions were statistically compared, the left ear showed a significant increase in TTS at 4 000 Hz for the last session and a significant decrease in TTS at 500 Hz and 1 000 Hz. The right ear had significantly greater TTS at 1 000 Hz and at 4 000 Hz for

the last exposure with an increment in threshold shift apparent at all test frequencies. The results are graphically displayed in Fig. 2.

Five months after the last rock and roll session, pure tone thresholds were again measured on all subjects. Subjects reported that they had not been exposed to any further discotheque sessions, although most listened to loud radio music at home. All subjects had hearing within the normal range with air conduction thresholds equal to bone conduction thresholds. When initial tests were compared with the follow-up audiograms, it was evident that the hearing sensitivity of most of the teen age subjects had returned to original levels within ± 5 dB. One subject, however, had his hearing threshold diminished by 10 dB to 15 dB at four frequencies in the left ear and at one frequency in the right ear. This particular subject was not one of the several subjects who showed large TTS on post-exposure tests.

DISCUSSION AND CONCLUSION

Results of this study closely agree with other reports in the literature on TTS due to exposure to rock and roll. Although this project forfeited the more rigorous control of laboratory experiments in exchange for subjects who voluntarily exposed themselves to highly amplified pop music for several hours in repeated sessions, the TTS found for individuals was similar to that published by Jerger & Jerger (1970) for two groups of performers tested one hour after exposure. Average TTS in this study was slightly greater than Rintelmann

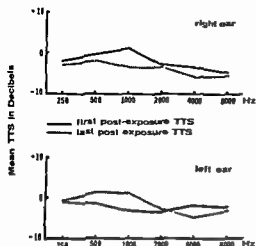


Fig 2 Comparison of post-exposure TTS for first and last rock and roll sessions. The 0 line represents pre-exposure threshold. Deviation above 0 represents improved sensitivity. Deviation below 0 indicates decreased sensitivity.

and his co-workers (1972) measured at 30 min post exposure to intermittent rock-and-roll music. However, Rintelmann's subjects were exposed for only one hour.

Sound pressure levels measured during the rock-and-roll sessions differed little from those reported by other investigators for numerous age groups (Lebo & Oliphant, 1968, Rintelmann & Borus 1968, Speaks & Nelson, 1968, Lipscomb, 1969). Since the sessions in this study were held in the open air, one might have expected that TTS would have been less than that previously reported. However, this was not the case.

Of particular interest were the differences in TTS between the two ears at the same frequencies. Jerger's (1970) study showed similar differential effects in the TTS in the two ears of the performers. Ward (1967) pointed out that the same ear may also exhibit different susceptibilities to different frequency bands. In this study the average TTS was greater at 250 Hz and 500 Hz in the right ear. The left ear had more TTS than the right ear at 1 000 Hz and 2 000 Hz, and the right ear had more TTS at 4 000 Hz and 8 000 Hz than the left ear. It is difficult to determine

the reason for such differences in susceptibility. One might hypothesize that the microscopic physical variations between the two ears in the position or angle of the cochlear duct relative to the oval window could be responsible. Such a difference might cause the fluid pressure waves in the inner ear to stress the sensory structures at slightly different points.

The fact that this project followed the subjects through a series of weekly exposures to rock-and-roll permitted a comparison of TTS between initial and final sessions. Although individual hearing recovered between sessions, the average TTS generally became worse over the succession of weekly exposures. While the mean right ear TTS was greater for the final exposure at all frequencies, left ear TTS for the final session was greater than after the first session only at frequencies above 2 000 Hz. Part of the explanation for this result might be that the left ear suffered more initial TTS than the right ear in the lower frequencies. The smaller TTS after the final exposure might indicate that the inner ear had already undergone physiological changes so that lateral inhibition along the cochlear partition was affected, making hearing appear more sensitive to clinical testing. The mean left ear TTS at 1 000 Hz was initially greater than that of the right ear at the same frequency. With the higher frequencies in the mid lower basal turn of the cochlea greatly depressed, the inner ear response to lower frequencies farther along the basilar membrane might have been protected or less inhibited by lateral inhibition.

Although the relationship between noise-induced temporary and permanent threshold shifts is still not clear, the fact that the hearing of most teenagers in this study returned to original levels of sensitivity does not rule out possible damage to the sensory elements of the inner ear. Histological examinations of both human and animal ears have shown that large numbers of hair cells may be missing along the organ of Corti when hearing thresholds are reported to be clinically normal. Re-

cent data from studies of the cochleae of chinchillas and monkeys exposed to loud noises to produce TTS, revealed that the inner ear underwent observable permanent changes in spite of the recovery of normal hearing sensitivity (Mills et al, 1970; Miller et al, 1971; Pinheiro et al, 1972).

There was no way to determine whether the permanent decrease in hearing sensitivity found at follow up in one of the subjects was due to the repeated rock and roll exposures. Although no other explanation could be found there was no difference greater than 5 dB between his initial audiogram and his last pre-exposure hearing test.

SUMMARY

The hearing sensitivity of teen age subjects exposed to several hours of rock and roll music in repeated weekly sessions recovered to pre-exposure levels in spite of significant post-exposure threshold shifts TTS for the two ears differed for the same frequencies. There was generally greater TTS for the final exposure than for the initial exposure. Research suggests that recovery of clinically normal hearing after TTS does not preclude permanent damage to cochlear sensory structures.

ZUSAMMENFASSUNG

Diese Untersuchung befasst sich mit dem Gehörorganismus von Teenagern, die sich freiwillig des öfteren zum Anhören von lautverstärktem Rock und Roll zur Verfügung gestellt haben. Vor Beginn und 30 Minuten nach jeder der 8 wöchentlichen Musikexperimente wurde eine durchschnittliche Lautstärke von 110 dB bis 115 dB gemessen. Der Hörfrequenzbereich wies bedeutende vorübergehende Veränderungen im Hörfrequenzbereich auf, wurden bei allen Versuchspersonen festgestellt; hauptsächlich in den hohen Frequenzen. Die Gehörsensitivität erholte sich zwischen den wiederholten Experimenten und eine Nachuntersuchung nach 5 Monaten ergab, dass das Gehör aller mit einer Ausnahme auf den Zustand vor dem Ganzexperiment zurückgegangen war. Verschiedene Effekte auf beide Ohren in derselben Frequenz wurden festgestellt.

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ACTION OF CHOLINERGIC AND ANTICHOLINERGIC DRUGS AT THE CROSSED OLIVOCOCHLEAR BUNDLE-HAIR CELL JUNCTION

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Abstract Both cholinergic and anticholinergic drugs introduced into the scala tympani of guinea pigs were found to block the effects induced by electrical stimulation of the crossed olivocochlear bundle. Strychnine, *d*-tubocurarine and decamethonium were most potent in this activity. Eserine did not potentiate the effects of electrical stimulation of the crossed olivocochlear bundle but did potentiate the effects of acetylcholine and in high concentrations blocked the crossed olivocochlear bundle. Acetylcholine (247 μ M) in combination with eserine (20 μ M) mimicked the effects of the crossed olivocochlear bundle by producing an augmentation of the cochlear microphonics and an inhibition of the action potential. The acetylcholine (247 μ M)-eserine (20 μ M) combination also produced a reversible blockade of the crossed olivocochlear bundle and the mimicking effects of subsequent perfusions with the drug combination. The mimicking effect of the acetylcholine (247 μ M)-eserine (20 μ M) combination was blocked by *d*-tubocurarine. These results further extend the evidence which has accumulated towards proving that acetylcholine is the transmitter substance of the crossed olivocochlear bundle.

The crossed olivocochlear bundle is an efferent inhibitory tract of nerve fibers which originate in the accessory nucleus of the superior olivary complex and form synaptic junctions at the base of the hair cells of the contralateral cochlea (Smith, 1967). Electrical

stimulation of these fibers results in an inhibition of the compound action potential of the auditory nerve (Galambos, 1956), an augmentation of the cochlear microphonics (Fex, 1959), an increase in the positive summing potential (Konishi & Kelsey, 1970), and a slow negative change in the endocochlear potential (Fex, 1967a). Cholinesterase (Schuknecht et al., 1959) and nerve endings containing synaptic vesicles (Smith, 1967) have been associated with these efferent fibers in the cochlea. Therefore, it seems that a transmitter substance may be released by the crossed olivocochlear bundle nerve endings.

Many laboratories have tested the hypothesis that acetylcholine or gamma-aminobutyric acid is the inhibitory transmitter substance (as cited in Guth & Bobbin, 1971). Conflicting results were obtained when iontophoretic drug application was used (Gisselsøen, 1960; Tanaka & Katsuki, 1966). Other pharmacological studies appear to have been hampered by a blood cochlear barrier which prevented drugs from reaching the site of the efferent nerve endings. Thus cholinergic agents applied intra-arterially evoked an inhibition of the action potential (Brown et al., 1969; Amaro et al., 1966), a result which is similar to that resulting from electrical stimulation of the crossed olivocochlear bundle. Anticholinergics applied in the same manner prevented the drug-induced response (Amaro et al., 1966; Dargneault & Brown, 1966) but

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did not prevent the crossed olivocochlear bundle induced response (Guth & Amaro, 1969, Brown et al., 1969)

The technique of placing test solutions containing *d* tubocurarine in perilymphatic space of the scala tympani has been shown to block the cochlear responses to electrical stimulation of the efferent nerve fibers (Fex, 1968, Konishi, 1972). Thus, the intracochlear application of drugs appears to by-pass vascular barriers to the nerve-hair cell junctions. Therefore, this study was designed to test whether acetylcholine is the transmitter substance of the crossed olivocochlear bundle through the use of the scala tympani perfusion technique to determine whether acetylcholine duplicates the effects of electrical stimulation of the crossed olivocochlear bundle, the influence of known acetylcholine antagonists on the effects of electrical stimulation of the crossed olivocochlear bundle, and the influence of eserine, an inhibitor of cholinesterase, on both the acetylcholine induced and crossed olivocochlear bundle induced effects. A preliminary report of the data presented herein has been published (Bobbin & Konishi, 1971)

METHODS

Pigmented guinea pigs (200–300 g) of either sex were used. They were anesthetized with Sodium Pentobarbital (Nembutal[®], Abbott, 25–30 mg/kg i.p.) The jugular vein was cannulated for intravenous drug administration. The trachea was also cannulated. In all animals the right auditory bulba was exposed and opened. The middle ear muscle tendons were sectioned in most animals. After this surgical procedure the animals were placed in an acoustical and electrical shielded room. Galamine triethiodide (Flaxedil[®], Davis & Geck, 8 mg/kg, i.v.) was administered and the animal maintained by artificial respiration to prevent movements of the animal.

The differential electrode technique was employed at the basal turn of the cochlea

to record sound evoked responses (Tasaki et al., 1952). Tone bursts of 6 kHz and 600 Hz with durations of 10 msec and rise and fall times of 0.5 msec were delivered to the external auditory canal through a closed system. Both the action potential and cochlear microphonics were recorded in response to the 6 kHz tone burst whereas only the cochlear microphonics were recorded in response to the 600 Hz tone burst.

The endocochlear potential was recorded by a microcapillary electrode filled with 3 M KCl which was inserted into the scala media through the spiral ligament (Konishi et al., 1961). A chloride-coated silver wire placed on the intact neck muscles served as the reference electrode. The cerebellum was exposed and a part of it was suctioned away to uncover the floor of the fourth ventricle. Bipolar stimulating electrodes were placed under visual control between the facial genua on the floor of the fourth ventricle in order to stimulate the crossed olivocochlear bundle. The electrode was placed along the midline of the brain stem where the largest negative change in the endocochlear potential could be recorded. This negative change in the endocochlear potential (the crossed olivocochlear potential) has been described (Fex, 1967, 1967a, Konishi & Kelsey, 1970). The electrical stimulus was a train of impulses (pulse duration, 0.3 msec, pulse frequency, 400/sec, train duration, 100 msec, current, 0.08–0.20 mA) which preceded the tone bursts by 10 msec.

The cochlear potentials including the endocochlear potential were displayed on an oscilloscope and photographed by a Grass C₄ kymograph camera. The endocochlear potential was also recorded on a Beckman Type R chart recorder.

The methods used to perfuse the scala tympani with an artificial perilymph have also been cited and described elsewhere (Konishi & Kelsey, 1970). The artificial perilymph used was composed of NaCl 139 mM, KCl, 5.6 mM, CaCl₂, 2.2 mM, NaHCO₃, 21 mM, gassed

Table I Effect of perfusion of the scala tympani with artificial perilymph and acetylcholine (247 μ M) together with eserine (20 μ M) on the action potential (AP) and the cochlear microphonics (CM)

	No of animals	AP 6 kHz (%) ^a	CM 6 kHz (%)	CM 600 Hz (%)
Artificial perilymph	12	-18 \pm 3	-2 \pm 1	+9 \pm 2
Acetylcholine-eserine	4	-54 \pm 3	+24 \pm 2	+64 \pm 5

^a Results are presented as the mean percentage change from control together with the standard error. The data are taken from the first perfusion in each animal

with 95% O₂ 5% CO₂, with resultant pH of 7.2 Drug concentrations in the artificial perilymph are expressed in terms of the free base. The perfusion solutions were applied to the scala tympani at room temperature (approximately 25°C). The rate of perfusion was slow (2 μ l/min) so as to minimize any displacements of cochlear tissues. A constant duration of perfusion (10 min) was used. Before each perfusion several control observations were made over a period of at least 10 min to insure that all cochlear responses were stable.

The following drugs were studied: carbamylcholine chloride (carbachol), acetylcholine chloride, eserine sulfate, choline iodide, nicotine tartrate, *d*-tubocurarine chloride, strychnine sulfate, atropine sulfate (atropine), atropine methyl bromide (methylatropine), arecoline hydrobromide, hexamethonium chloride, and decamethonium bromide.

RESULTS

Action of artificial perilymph

The first perfusion of the scala tympani with artificial perilymph suppressed the action potential and the cochlear microphonics in response to 6 kHz and augmented the cochlear microphonics in response to 600 Hz (Table I). Subsequent perfusions had less of an effect on the action potential or the cochlear microphonics. These potentials tended to decrease in size as the number of perfusions increased.

Because the summing potential varied in

different preparations, it was not feasible to quantitate it.

The endocochlear potential was increased in a positive direction (5 mV) by the first perfusion with artificial perilymph. Subsequent perfusions produced less of an increase.

The degree of both the inhibition of the action potential and the augmentation of the cochlear microphonics produced by electrical stimulation of the crossed olivocochlear bundle changed in parallel with the change in the magnitude of the crossed olivocochlear potential. This was found to occur in all of the experiments to be described in this paper. Therefore, the crossed olivocochlear potential was chosen as the parameter to present as an evaluation of the activity of the crossed olivocochlear bundle.

The artificial perilymph solution increased the crossed olivocochlear potential approximately 24% during the first perfusion of the scala tympani (Table II). At the end of a second perfusion the average value of the crossed olivocochlear potential fell to 88% of control.

Action of acetylcholine and choline

Neither acetylcholine (247 μ M, 550 μ M) nor choline (550 μ M) produced changes in the action potential, cochlear microphonics, or endocochlear potential which could be distinguished from the effects of the artificial perilymph.

Acetylcholine 247 μ M produced a slight inhibition of the crossed olivocochlear po-

Table II *Effect of cholinergic and anticholinergic drugs perfused through the scala tympani on the magnitude of the crossed olivocochlear potential (COCP)*

1st perfusing agent	Magnitude of the COCP ^a			No of animals
	During 1st perfusion	Post 1st perfusion	Post 2nd perfusion	
	4-6 min	0-10 min	0-10 min	
Artificial perilymph	123 (106-134)	102	88	4
d Tubocurarine 5 μ M	24 (20-27)	0	59	2
Strychnine 10 μ M	0 (0-0)	0	59	2
D-camethonium 25 μ M	55 (53-57)	0	77	2
Hexamethonium 550 μ M	88 (64-72)	43	76	2
Methylatropine 50 μ M	23 (20-25)	0	84	2
Atropine 200 μ M	45 (44-46)	19	72	2
Eserine 800 μ M	38 (17-40)	21	81	4
Nicotine 1 mM	68 (60-75)	— ^b	86 ^b	3
Acetylcholine 546 μ M	54 (50-58)	21	89	3
Choline 550 μ M	54 (45-67)	13	80	3
Acetylcholine 247 μ M and Eserine 20 μ M	0 (0-0)	0	93	3
Acetylcholine 54 μ M and Eserine 20 μ M	22 (14-29)	0	75	2
Carbachol 50 μ M	57 (55-59)	26	87	2

^a The magnitude of the COCP is presented as a mean per cent of control. The range for the first (4-6 min) value is included.

^b The second perfusion agent was artificial perilymph and was started 10 min after the end of the 1st perfusion.

^c No second perfusion was carried out after the nicotine recovery recorded in 30-50 min.

tential (25% in 10 min in two animals). At 546 μ M acetylcholine produced a greater inhibition of the crossed olivocochlear potential which was identical to that produced by choline (550 μ M) (Table II). Thus, choline and acetylcholine were equipotent on a molar basis.

Action of eserine

The effects of eserine (20 μ M, two experiments) could not be distinguished from the effects of the artificial perilymph. With higher concentrations of eserine (800 μ M) the pH of the artificial perilymph changed (pH

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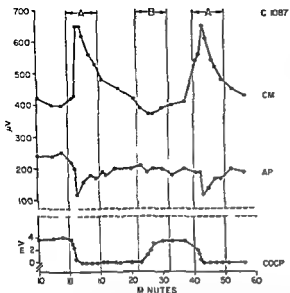


Fig 1 Effect of perfusing the scala tympani with the acetylcholine (247 μ M)-eserine (20 μ M) combination (A) and artificial perilymph (B) CM: Cochlear microphonics evoked by 600 Hz tone burst (67 dB SPL) recorded without electrical stimulation of the crossed olivocochlear bundle AP Action potential evoked by a 6 kHz tone burst (82 dB SPL) recorded without electrical stimulation of the crossed olivocochlear bundle COCP Crossed olivocochlear potential evoked by electrical stimulation of the crossed olivocochlear bundle Note that the effect of this electrical stimulation of the crossed olivocochlear bundle on the action potential and cochlear microphonics has been omitted

crossed olivocochlear potential (2 animals) Nicotine (1 mM) showed just a slight inhibitory effect on the crossed olivocochlear potential (Table II) Carbachol (50 μ M) produced

an inhibition of the crossed olivocochlear potential that was slightly less than that produced by the same molar concentration of acetylcholine (54 μ M) when combined with eserine (20 μ M) (Table II)

Action of several anticholinergics

None of these agents produced changes in the cochlear microphonics, action potential, or endocochlear potential which could be distinguished from the effects of the artificial perilymph

Strychnine (10 μ M) and *d* tubocurarine (5 μ M) were the most potent (on a molar basis) in inhibiting the crossed olivocochlear potential (Table II) The two bis-onium compounds, hexamethonium and decamethonium, were found to be less effective with decamethonium being 20 times more potent than hexamethonium (Table II) The quaternary atropine, methylatropine, was found to be four times as potent as the tertiary atropine in inhibiting the crossed olivocochlear potential (Table II) The time course of the inhibition and recovery of the crossed olivocochlear potential after the application of inhibitory agents is illustrated by methylatropine in Fig 2

DISCUSSION

That the events following electrical stimulation of the crossed olivocochlear bundle are mediated by the release of acetylcholine from the bundle's nerve endings has been proposed

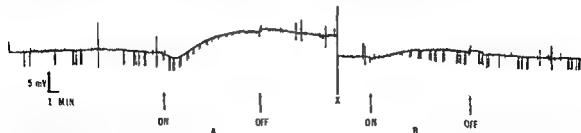


Fig 2 Changes in the endocochlear potential and in the negative deflections produced by electrical stimulation of the crossed olivocochlear bundle (crossed olivocochlear potentials) produced by perfusion of the scala tympani (during the on-off period) with

methylatropine (50 μ M) (A) and artificial perilymph (B) X manual adjustment of the recording pen Deflections with an upward (positive) component represent electrical artifacts Time scale and voltage calibration in lower left corner

by several groups of workers. This hypothesis gained credence when it was shown that curare and atropine applied intracochlearly (Konishi, 1972, Fex, 1968) and hemicholinium administered intravascularly (Guth & Amaro, 1969, Brown et al, 1969) blocked the crossed olivocochlear bundle. This study has extended the group of anticholinergics which block the crossed olivocochlear bundle to include decamethonium, hexamethonium and methylatropine.

Strychnine has been reported to block the crossed olivocochlear bundle (Desmedt & Monaco, 1962, Fex, 1967a). We have confirmed this observation and shown that its potency was about equal to curare. That strychnine possesses anticholinergic properties has been demonstrated (Feldberg & Vartiainen, 1934, Lanari & Lucio, 1939). Thus, the blocking ability of strychnine confirms the susceptibility of the crossed olivocochlear bundle to anticholinergics.

The enzyme, acetylcholinesterase, which may be used for termination of the action of the proposed transmitter substance has been located in association with these efferent fibers (Schuknecht et al, 1959). However, we were unable to demonstrate a potentiation of the crossed olivocochlear bundle by eserine which inhibits cholinesterase. The cholinesterase levels in the cochlea decline upon efferent fiber but not hair cell degeneration (Schuknecht et al, 1959). This may be interpreted to mean that the enzyme has a location on the crossed olivocochlear bundle nerve endings and not "post synaptic" on the hair cells. This localization is similar to that in the autonomic ganglia (Koelle & Koelle, 1959) where it is difficult to demonstrate an eserine potentiation of the neural response (Feldberg & Vartiainen, 1934). This action of eserine has been taken to mean that the termination of the transmitter action is by means of diffusion away from the receptor site rather than by hydrolysis due to the cholinesterase (Emmelin & MacIntosh, 1956). Since cholinesterase inhibitors would not prevent the dif-

fusion process, then an accumulation of transmitter substance and a resultant potentiation of nerve firing would not be observed. Therefore, our results with eserine do not negate the hypothesis that acetylcholine is the transmitter substance of the crossed olivocochlear bundle.

The curare like action of high doses of eserine found in this study has been documented for other known cholinergic synapses (Feldberg & Vartiainen, 1934, Eccles & MacFarlane, 1949).

If acetylcholine is the transmitter substance exogenously applied acetylcholine should mimic the effects of nerve stimulation. We examined the effects of acetylcholine and several agents which possess acetylcholine like activity: carbachol, nicotine, choline, arecoline, and decamethonium. All of these except arecoline produced an inhibition of the crossed olivocochlear bundle but did not mimic the effects of nerve stimulation at the concentrations used. Choline was found to be equipotent to acetylcholine in producing this effect. This suggested that all of the acetylcholine applied was hydrolysed by the cholinesterase in the cochlea and arrived at the blocking site as choline. This was confirmed by the addition of eserine to acetylcholine and choline solutions. It was found that the eserine potentiated the inhibitory effect of acetylcholine more than ten times but not that of choline.

Classically, acetylcholine and acetylcholine like agents demonstrate blocking activity at known cholinergic sites (Feldberg & Vartiainen, 1934, Thesleff, 1955, Paton & Savini, 1968, Nastuk & Gissen, 1966, Paton & Zaimis, 1949, Barlow, 1964). The rate of drug delivery ($2 \mu\text{l}/\text{min}$) used in this study was so slow that apparently no acetylcholine reached the blocking site before hydrolysis by cholinesterase. We demonstrated a mimicking response only by combining a high concentration of acetylcholine with eserine. The addition of eserine insured a delivery of acetylcholine molecules. The large concentration of acetylcholine increased the rate of delivery

without an increase in the perfusion rate. These results are in agreement with the classical demonstrations that the mimicking effects of acetylcholine are highly dependent on concentration and a fast rate of delivery to cholinergic sites (Brown et al., 1936, Riker, 1966). Similarly, concentrations higher than those used in this study will probably have to be used to demonstrate a mimicking activity with the acetylcholine like agents. We may have also explained why others failed to demonstrate a mimicking activity with acetylcholine applied into the cochlea (Sohmer & Feinmesser, 1963).

Parallel with the mimicking response to acetylcholine, a blockade of the crossed olivocochlear bundle occurred. This action of acetylcholine may be similar to that classically labeled a "polarization blockade" (Paton & Perry, 1953, Barlow, 1964). Electrical stimulation of the crossed olivocochlear bundle is thought to result in a hyperpolarization of the hair cells which causes an augmentation of the cochlear microphonics and an inhibition of the action potential (Fex, 1967). A hyperpolarization of the hair cells by exogenously applied acetylcholine would produce a blockade of the crossed olivocochlear bundle by preventing additional hyperpolarization due to the nerve released transmitter.

The hyperpolarization of the hair cells is reflected by a negative change in the endocochlear potential called the crossed olivocochlear potential (Fex, 1967). Our inability to detect a negative change in the endocochlear potential recording does not negate the "polarization" hypothesis. Thus, the artificial perilymph may have increased the endocochlear potential through a mechanism independent of the hair cell potential. This increase in the endocochlear potential by the artificial perilymph may have masked any changes in the hair cell potential which would have otherwise been reflected and measured in the endocochlear potential recording. However, the augmentation of the cochlear microphonics and inhibition of the action potential evoked

by the acetylcholine is evidence for a "polarization" blockade of the crossed olivocochlear bundle.

The mimicking response evoked by acetylcholine faded rapidly with the continued application of the drug. Also the cochlear tissues did not respond to further applications of acetylcholine. Thus, it appears that the continued presence of acetylcholine, which was insured by the inhibition of cholinesterase by eserine, resulted in an "inhibition" of the sites initially activated by the acetylcholine. This type of acetylcholine response has been documented for other cholinergic systems and labeled "desensitization" (Barlow, 1964, Thesleff, 1955).

The site of action of all of the agents used in this study is unknown. The order of potency in inhibiting the crossed olivocochlear potential was the reverse of that found on unmyelinated nerve fibers where nicotine was found to be the most potent agent (Armstrong & Ritchie, 1961). Also the action potential was not reduced except by the acetylcholine (247 μ M)-eserine (20 μ M) combination. If the agents were acting in a non specific (procaine like) manner to block conduction along the unmyelinated portions of the nerve fibers in the cochlea, both the action potential and the crossed olivocochlear potential would be blocked. Thus, it appears that the site of action of the majority of the drugs was not on the nerve fibers.

The effects of both the exogenously applied acetylcholine and the crossed olivocochlear bundle were blocked by curare. This suggests that the inhibitory transmitter substance and the exogenously applied acetylcholine were acting at the same curare sensitive sites.

In conclusion we have shown that the action of the drugs studied are compatible with the hypothesis that acetylcholine is the transmitter substance of the crossed olivocochlear bundle. Several criteria must be satisfied before a chemical is regarded as a transmitter substance (McLennan, 1963). In this study we have demonstrated the mimicking of nerve

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stimulation by exogenously applied acetylcholine, and the blockade of exogenously applied acetylcholine by the same agents which block nerve stimulation. Others have shown the presence of cholinesterase (Schuknecht et al., 1959) the release of acetylcholine upon crossed olivocochlear bundle stimulation (Norris 1972) and the presence of cholineacetylase which synthesizes acetylcholine (Guth & Jasser, 1973).

ACKNOWLEDGMENT

The authors thank Miss Elizabeth Kelsey for her technical assistance.

ZUSAMMENFASSUNG

Wie gezeigt werden konnte bewirkt ein Introdizieren von sowohl cholinergischen wie anticholinergischen Drogen in die Scala tympani von Meerschweinchen eine Blockade der Effekte die bei elektrischer Reizung des gekreuzten Olivocochlear-Bundels ausgelöst werden. Strychnin, d-Tubocurare und Dekamethonium wirkten in dieser Hinsicht am stärksten. Eserin verstärkte nicht die durch elektrische Reizung des gekreuzten Olivocochlear-Bundels ausgelösten Effekte, potenzierte jedoch die Einwirkung von Acetylcholin und blockierte bei hoher Konzentration das gekreuzte Olivocochlear-Bündel. Durch Acetylcholin ($247 \mu\text{M}$) in Kombination mit Eserin ($20 \mu\text{M}$) wurden die Effekte des gekreuzten Olivocochlear-Bundels gleichsam imitiert, d.h. es ergab sich eine Erhöhung der Cochlearmikrophonie und eine Hemmung (Inhibition) des Aktionspotentials. Die Acetylcholin ($247 \mu\text{M}$) — Eserin ($20 \mu\text{M}$) — Kombination löste auch eine reversible Blockade des gekreuzten Olivocochlear-Bundels und der Imitations-Effekte bei subsekutivem Spritzen mit dieser Drogenkombination aus. Der Imitations-Effekt der obigen Acetylcholin-Eserin-Kombination wurde durch d-Tubocurare blockiert. Diese Ergebnisse sind eine weitere Bestätigung dafür, dass Acetylcholin die Transmittersubstanz des gekreuzten Olivocochlear-Bundels ist.

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mit dem Modiolusteil des Nervus

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DAS PERIPHERE OLIVOCOCHLEARE BÜNDEL DER LABORATORIUMSRATTE

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Abstrakt Mit Hilfe der Darstellung der Acetylcholinesterase wurden die efferenten Fasern in der Cochlea der Laboratoriumsratte untersucht. Das Verteilungsmuster ist prinzipiell wie bei anderen Säugetieren. Unterschiede ergaben sich bei den intralaminaren Spiralbündeln und bei den direkten efferenten Fasern im Nervus cochlearis. Beide Faserarten waren mit dem Acetylcholinesterase-Nachweis nicht darstellbar.

Das olivocochleare Bündel (OCB) wurde zwar schon durch Rasmussen (1942) entdeckt, aber erst durch die Verwendung des Nachweises der Acetylcholinesterase (AChE) wurde eine einfache Möglichkeit eröffnet, die efferenten Fasern darzustellen (Schuknecht et al., 1959). Die zahlreichen seither mit dieser Methode erhobenen Befunde sind von Iurato und Mitarbeitern (1971) zusammengestellt worden. Bei der Ratte wurden zwar in jüngster Zeit von Brown & Howlett (1972) die zentralen Abschnitte des OCB untersucht, über den peripheren Teil liegen jedoch bisher nur elektronenmikroskopische Ergebnisse (Iurato, 1962) vor. Die vorliegende Untersuchung ist im Hinblick auf die geplante operative Durchtrennung des OCB bei der Ratte entstanden. Die funktionelle Bedeutung des efferenten Systems ist bis heute noch nicht hinreichend geklärt (Klinke et al., 1971). Eine Funktion unter physiologischen Bedingungen wird von manchen Autoren überhaupt bestritten (Pfalz, 1969; Igarashi et al., 1972). Eine genaue morphologische Untersuchung des efferenten Sys-

tems erscheint bei einem wichtigen Versuchstier, wie der Laboratoriumsratte, angebracht.

MATERIAL UND METHODE

Albinoratten von einem Gewicht zwischen 250 und 400 g wurden mit Äther und Chloralhydrat anästhesiert. Danach perfundierten wir vom Herzen aus mit 5%iger Formollosung (Phosphatpuffer pH 7,4). Die isolierten Schläfenbeine wurden zwei Tage nachfixiert und dann in EDTA entkalkt. Von den weitgehend isolierten Cochleae fertigten wir 10 µ dicke Gefrierschnitte an. Diese wurden nach 12stündiger Aufbewahrung im Kühlschrank nach Karnovsky und Roots sehr lange (24 Stunden) inkubiert. Als Substrat für die AChE diente Acetylthiocholinjodid. Versuchsweise wurden einige Schnitte mit Propionylthiocholinjodid inkubiert, da nach Gruber et al. (1971) und nach Eranko (1973) die AChE bei der Ratte auch dieses Substrat spaltet. Zur Sicherung der Spezifität der Reaktion fügten wir dem Inkubationsmedium 10^{-4} M Iso-Ompa zu, um die unspezifische Cholinesterase zu hemmen. Die meisten Schnitte wurden mit Hämatoxylin schwach gegengefärbt.

ERGEBNIS

Die AChE läßt sich in den efferenten Fasern des Innenohres und in den Perikarya der

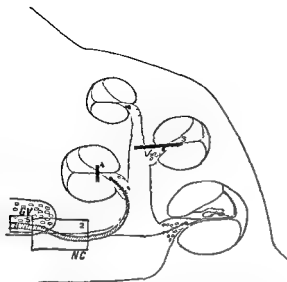


Abb 1 Schema der Cochlea schraffiert dargestellt ist das olivocochleare Bündel GV, Ganglion vestibulare NC Nervus cochlearis Die Zahlen 2 3 4 und 5 verweisen auf die folgenden Abbildungen

Ganglienzellen des Ganglion vestibulare und des Ganglion spirale nachweisen. Bei der langen Inkubationszeit, die wir benutzten, zeigen die efferenten Fasern eine starke und konstante Reaktion. In den Perikarya der Ganglienzellen variiert die Reaktionsstärke. An den Spiralganglienzellen konnten wir diese Erscheinung beschreiben (Seidler et al., 1972, Firbas et al., 1973). Von anderen sensiblen Ganglienzellen ist der Gehalt an AChE schon früher beschrieben worden (Giacobini, 1959, Liedel, 1971, Gwyn & Flumerfelt, 1971).

Da die efferenten Fasern bis zu ihren Endigungen gut darstellbar sind, läßt sich der Verlauf genau verfolgen. Die efferenten Fasern liegen zuerst im Nervus vestibularis direkt, primär im Nervus cochlearis verlaufende Fasern fanden wir bei der Ratte nicht. Vom Nervus vestibularis ziehen die efferenten Fasern in der Oort'schen Anastomose an den Nervus cochlearis (Abb 1 und 2). Der größere Teil der Fasern biegt nach apical ab und gelangt, den Cochlearisstamm verlassend, sofort zum Spiralganglion der oberen Basalwindung. Diese Fasern haben in der Fortsetzung keinen

Kontakt mit dem Modiolusteil des Nervus cochlearis.

Der Rest der Fasern, der zum Spiralganglion der unteren Basalwindung zieht, durchsetzt fächerförmig in mehreren Bündeln den Nervus cochlearis beim Eintritt in den Modiolus (Abb 1 und 2). Vom intraganglionären Spiralbündel laufen radiale Fasern zwischen den beiden Knochenlamellen der Lamina spiralis ossea zum Cortischen Organ (Abb 3). Ausgesprochen spiralförmige Fasern in der Lamina spiralis, wie sie Nomura & Schuknecht (1965) beim Menschen fanden, konnten wir nicht nachweisen. An Radialschnitten ist zu erkennen, daß die AChE positiven Fasern von der tympanalen Lamelle zur vestibulären Lamelle aufsteigen und durch die Habenula perforata zum Cortischen Organ gelangen. An Querschnitten durch die Lamina spiralis sind die AChE-positiven Fasern zu erkennen (Abb 4).

Im Bereich des Cortischen Organs sind efferente Fasern in den spiralförmigen und radiären Tunnelbündeln nachweisbar. Die efferenten Nervendigungen an den äußeren Haarzellen wurden von uns nicht im einzelnen registriert. Es steht aber auch hier das bekannte Muster fest. Im apicalen Bereich der Cochlea und jeweils an der dritten Reihe der äußeren Haarzellen sind geringere Depots von Reaktionsprodukten abgelagert.

Bei starkerer Vergrößerung ließ sich fallweise erkennen, daß die Reaktionsprodukte in Doppelkonturen entlang von Nervenfasern vorliegen (Abb 5). Das steht in Einklang mit den elektronenmikroskopischen Befunden von Nakai (1972). Dieser fand AChE-Aktivität bei markhaltigen Fasern an der Axoplasmamembran. An den Bildern von Nakai kann man feststellen, daß die AChE bei den markhaltigen Fasern nicht in die Umgebung der Fasern diffundiert. Obwohl wir mit langer Inkubationszeit arbeiteten, scheint durch die Fixation der Präparate die Diffusion von AChE auch bei den unmyelinisierten Fasern weitgehend vermieden worden zu sein. Der Gehalt an AChE in den Spiralganglienzellen ist offensichtlich geringer als in den efferenten Fasern.

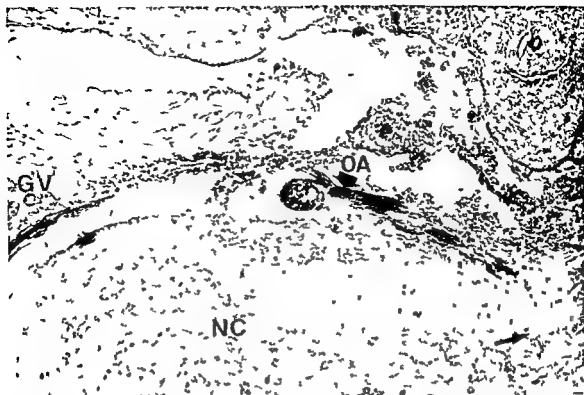


Abb 2 Olivocochleares Bündel im Übergang vom Nervus vestibularis zum Nervus cochlearis GV Ganglion vestibulare NC Nervus cochlearis OA

Ortsche Anastomose Der Pfeil verweist auf ein efferentes Bündel zur unteren Basalwindung

Die efferenten Fasern zeigen schon nach 2 stündiger Inkubation deutliche Reaktionen während die Spiralganglienzellen noch ungefarbt sind. Im Gegensatz zu Brown & Howlett (1972) konnten wir bei gleichen Versuchsbedingungen immer vergleichbare Resultate erzielen.

DISKUSSION

In den wesentlichen Merkmalen ist der Verlauf der peripheren AChE positiven (efferenten) Fasern bei der Ratte ähnlich wie bei den anderen bisher untersuchten Säugetieren: Katze (Rasmussen 1946, 1953; Gacek et al 1965), Kaninchen (Rossi & Cortesina 1965), Mensch (Gacek 1961; Nomura & Schuknecht 1965; Ishii et al 1967), Meerschweinchen (Terayama et al 1969; Terayama & Yamamoto 1971) und Totenkopffaffen (Ishii et al 1967). Auffallende Unterschiede sind nach unseren

Befunden keine AChE positiven Fasern, die mit dem Stamm des Nervus cochlearis herangeführt werden. Nach Ishii et al (1967) gehören solche Fasern nicht zum olivocochlearen Bündel. Auffallend war bei der Ratte auch das Fehlen spiralig verlaufender AChE positiver Fasern in der Lamina spiralis ossea. Nomura & Schuknecht (1965) konnten spiralige Bündel in großer Zahl bei Katze und Mensch darstellen. Es scheinen also beachtliche Artunterschiede im Verteilungsmuster des OCB zu bestehen. Auch der Ursprung des OCB unterscheidet sich bei der Ratte von dem der Katze und des Meerschweinchens (Brown & Howlett 1972): es entspringt als einheitliches Bündel in den Kernen des Trapezkörpers.

Die Eigenschaften der in der Lamina spiralis ossea verlaufenden Spiralbündel (intralaminar Spiralbündel) werden von Johnsson & Hawkins (1972) ausführlich erörtert. Diese Autoren halten die intralaminaren Spiralfasern für



Abb 3 Tangentialschnitt durch die Cochlea NC ganglionares Spiralbündel CO Cortisches Organ Der Nervus cochlearis GS Ganglion spirale IGSB intra Pfeil verweist auf radiäre, intralaminare Fasern



Abb 4 Querschnitt durch die Lamina spiralis ossea Der Pfeil verweist auf ein efferentes radial verlaufendes Nervenbündel

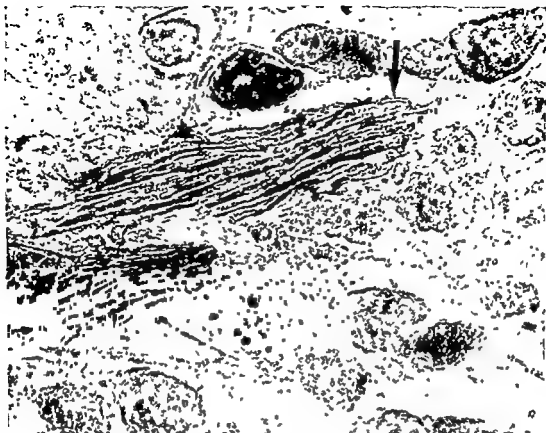


Abb 5 Ganglion vestibulare mit olivocochlearem Bündel. Mit dem Pfeil ist eine Doppelkontur entlang einer Nervenfasern angedeutet.

afferent. Ebenso umstritten ist die Bedeutung markhaltiger und markloser Fasern in diesem Bereich (Terayama & Yamamoto, 1971). Wie eingangs erwähnt ist die Diskussion um die Funktion der efferenten Fasern im Cortischen Organ nicht zu Ende. Neue pharmakologische Befunde lassen es als sicher erscheinen, daß zumindest ein Erregungsstoff der Fasern Acetylcholin ist (Galley et al, 1971, Bobbin & Konishi, 1971, Konishi, 1972, Klinke et al, 1973). Der Nachweis des Ferments Acetylcholinesterase steht also in sinnvollem Zusammenhang mit dieser Transmittersubstanz.

SUMMARY

The efferent olivocochlear bundle in the rat was demonstrated by histochemical localization of acetylcholinesterase. The distribution of the peripheral

olivocochlear bundle is similar to that of other mammals. Differences exist in the direct efferent cochlear fibres and in the intralaminar spiral fibres. Nestlé of these fibre types could be demonstrated in the rat with help of acetylcholinesterase activity.

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THE SIGNIFICANCE OF PERIPHERAL VISION IN THE PERCEPTION OF MOVEMENT

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Abstract Certain significant differences have been shown to occur in the character of the optokinetic response of a subject gazing actively and passively at a moving striped drum which suggest that different nervous mechanisms subservise each. Image motion across the retina appears to be the stimulus inducing the passive variety and is moreover largely responsible for the illusory sensations of self rotation. This same mechanism may be invoked by way of explanation of the phenomenon of reversed optokinetic nystagmus. The occurrence of this phenomenon in patients with non congenital as well as congenital nystagmus lends support to this explanation. Eye movements induced in the normal eye of subjects with unilateral ophthalmoplegia following stimulation of the parietic eye provide additional support for the contention that peripheral vision contributes to the control of normal ocular movements.

In the past investigations in man aimed at the elucidation of the physiological mechanisms subserving the control of normal ocular movements have for the most part involved studies of one kind or another concerned with optic fixation upon a single luminous moving target perceived either in total darkness or against a homogenous background. The conclusions derived from such studies may be questioned on the grounds that the experimental situation is an unusual one in that normally a moving target is viewed against a stationary background. Consequently as the eyes follow the target the images of the background will traverse the retinae in the opposite direction in a manner which will tend

to oppose movement. It follows that two separate and distinct channels of information must be transmitted to the brain one derived from the fovea and the other from the peripheral retina.

How this information is integrated at a cortical level is obscure but it would be surprising if the peripheral component were not used to advantage as a controlling mechanism aiding stability of eye movements. In what follows a series of investigations will be described in which it has been shown that peripheral vision can exert a significant and important influence upon ocular movement. These investigations have for the most part been concerned with observations of optokinetic nystagmus. For this purpose a striped revolving drum of the kind shown in Fig 1 was used. The drum is made of black material lined at intervals of 20 degrees with white vertical stripes. Provision is made for good illumination of the interior of the drum by means of a light placed above the subject's head. The drum itself is servo mechanically controlled and can be rotated at angular velocities up to 120°/sec.¹ The resultant eye movements were recorded electro-nystagmographically and displayed upon a conventional pen recorder using d.c. amplification.

Typical recordings of optokinetic nystagmus are shown in Fig 2. In the upper tracing the



Fig 1 Optokinetic drum. The drum is servo-mechanically controlled and may be rotated at velocities up to 120°/sec.¹

subject was instructed to deliberately follow the stripes of the drum as they came into view eliciting what might be termed an "active" form of optokinetic nystagmus. In the lower tracing the subject gazed without conscious effort at the moving stripes producing a "passive" form of optokinetic nystagmus. Although on cursory examination the wave form of the nystagmus under these two conditions may seem to be identical consisting of a slow component in the direction of rotation of the drum and a fast component in its opposite, certain significant points of difference are apparent. Thus in both tracings the arrows shown provide an indication of points at which drum direction was abruptly reversed. With "active" optokinetic nystagmus this results in a deviation of the eyes in the direction of rotation of the drum with the fast component returning the eyes towards the midline. In the case of "passive" optokinetic

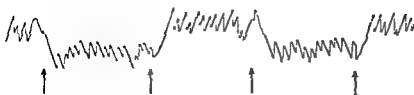
nystagmus on the other hand, the eyes are deviated in the direction of the fast component of the nystagmus and it is the slow component which returns the eyes towards the midline.

These findings have a significant bearing upon the controversy which has existed for many years upon the mechanism of optokinetic nystagmus. Thus Rademaker & Ter Braak (1948) take the view that the slow phase is a fixation movement, whereas the rapid phase is a movement whereby the slow phase is rhythmically interrupted owing to what they term the rhythmical activity of the cerebral mechanism. It was obviously the tacit acceptance of this mode of action that led Kestenbaum (1948) to write of train, synonymous with optokinetic nystagmus, in the following terms: "The genesis of this nystagmus is obvious. The eyes are kept fixed on an object in the scenery and therefore deviate backwards in slow motion, they then leave the object and quickly return to their previous position". In other words the form of optokinetic response envisaged by these authors would seem to accord with the upper tracing in Fig 2 of "active" optokinetic nystagmus. By contrast, Brucher (1964) and others in separate studies have commented upon the deviation of the eyes occurring in the direction of the fast component.

On this account Brucher was led to describe the fast component as the "prime movers" of the nystagmus. Borries (1926) similarly likens the fast component to a mechanism akin to the fixation reflex which he refers to as haptation whereby images of objects attracting our attention at the periphery are reflexly transferred to the macula.

This view assigns to the fast component a much higher cerebral function than that envisaged by Rademaker and Ter Braak in volving in all probability the frontal centres for voluntary gaze (Dix & Hood, 1971). Whether or not these observations may be applied to the fast component of "active" optokinetic nystagmus as defined here remains to be de-

ACTIVE OPTOKINETIC NYSTAGMUS



PASSIVE OPTOKINETIC NYSTAGMUS



SECONDS

Fig 2 Nystagmus tracings obtained with a subject following the stripes of the drum (Active optokinetic nystagmus) and gazing passively at the drum (Passive optokinetic nystagmus). Arrows indicate reversal of drum direction. Note deviation of eyes in the direction of the slow component of the nystagmus in the case of active optokinetic nystagmus and in the direction of the fast component in the case of passive optokinetic nystagmus.

terminated but in any event it is the "passive" form of optokinetic nystagmus which best fits the description of both Brucher and Borries.

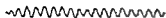
There exist however other essential points of difference between "active" and "passive" optokinetic nystagmus. In the "active" form the images of the stripes are fixed upon the fovea and consequently the eye velocity matches that of the drum velocity at all times. In fact, despite various claims to the contrary (Westheimer, 1954), it is possible in this way to elicit smooth tracking movements of the eyes to velocities matching drum velocities as high as $100^\circ/\text{sec}^{-1}$.

This however calls for considerable conscious effort and is difficult to maintain for any period of time even with quite low drum velocities. Instead, examination of recordings carried out under these conditions show that the eye movements fall into two types, those in which the eye velocity accurately matches that of the drum velocity and those in which the eye velocity is consistently inferior to that of the drum. In fact what appears to be happening is that despite conscious effort the nystagmic response repeatedly lapses from the "active" to the "passive" type response. In

the clinical situation therefore even though the patient may be instructed to follow the stripes of the drum it is in general the "passive" type which will be elicited by virtue of his inability to sustain the "active" response.

Now it is clearly apparent that the mechanism of this response must be quite different from that of "active" optokinetic nystagmus. Since the velocity of the eyes does not reach that of the drum there exists a velocity error between the two so that the images of the stripes continually traverse the retina. In consequence since the fovea is not dominantly involved as in the cases of "active" optokinetic nystagmus, the slow component of this type of nystagmus is not a true tracking movement, in which volition is an essential feature but rather it is the progression of the images of the stripes across the retina which appears to provoke the nystagmus. That this contribution from the peripheral retina is indeed a very potent stimulus may be judged from the fact that when foveal vision is absent as in patients with central scotomata the optokinetic response may be considerably enhanced (Hood, 1967). These observations have an important bearing upon

CONGENITAL



VESTIBULAR

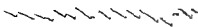


Fig 3 Spontaneous nystagmus of congenital and vestibular origin

the current interest being taken in the powerful illusory perception of self rotation evoked by an encircling optokinetic drum Dichgans & Brandt (1972) have studied this at length and shown that the illusion of movement induced in a stationary subject by means of an optokinetic drum is indistinguishable from rotation of the subject himself and thus they take as evidence of "neuronal vestibular convergence with a relatively long summation time for the visual input and a long time constant of decay after stimulation" Here again it can be shown that the illusion is heavily dependant upon image motion across the retina. Thus in the first place the illusion takes longer to develop and is much less vivid in

the "active" form of optokinetic nystagmus than in the "passive" Furthermore, if the subject is asked to fixate upon a stationary target placed close to the periphery of the moving optokinetic drum the illusion of turning is immediate and very considerably enhanced despite the fact that the act of optic fixation completely inhibits any nystagmic response. This undoubtedly results from the fact that in this situation since the eyes are stationary, retinal image movement is maximal. Precisely the same reasoning may be invoked by way of explanation of our repeated observation that patients presenting with a directional preponderance of optokinetic nystagmus consistently report that the illusion of movement is greatest with drum rotation evoking nystagmus in the direction in which the slow component is deficient, that is to say, the direction in which there exists the greatest difference between drum velocity and slow component velocity.

The above considerations have special relevance to the interpretation of certain unusual phenomena encountered in two groups of patients with particular ocular derangements.

The first group was concerned for the most part with patients presenting with so-called congenital nystagmus. The pathology of this condition is obscure its only manifestation being the presence of spontaneous, rapid and often large amplitude nystagmic movements of the eyes which give rise to relatively little incapacity. Although the character of the nystagmus may on occasions take on a vestibular appearance with a fast component in one direction and a slow component in the other it is more usually described as being pendular, having the form shown in Fig 3 in which for comparison is shown a tracing of true vestibular nystagmus.

One of the unusual features commonly held to be pathognomonic of congenital nystagmus is that such patients not infrequently exhibit a paradoxical reversed optokinetic response, that is to say with drum rotation say to the right which would normally evoke a nystagmus

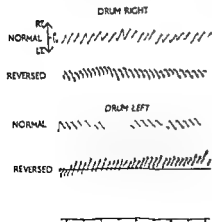
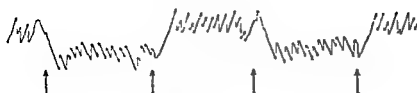
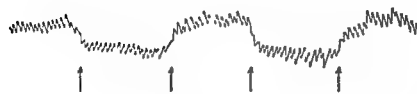


Fig 4 Reversed optokinetic nystagmus in subject with congenital nystagmus. Normal responses to the same stimuli are included for comparison.

ACTIVE OPTOKINETIC NYSTAGMUS



PASSIVE OPTOKINETIC NYSTAGMUS



SECONDS

Fig 2 Nystagmus tracings obtained with a subject following the stripes of the drum (Active optokinetic nystagmus) and gazing passively at the drum (Passive optokinetic nystagmus). Arrows indicate reversal of drum direction. Note deviation of eyes in the direction of the slow component of the nystagmus in the case of active optokinetic nystagmus and in the direction of the fast component in the case of passive optokinetic nystagmus.

terminated but in any event it is the "passive" form of optokinetic nystagmus which best fits the description of both Brucher and Borries.

There exist however other essential points of difference between "active" and "passive" optokinetic nystagmus. In the "active" form the images of the stripes are fixed upon the fovea and consequently the eye velocity matches that of the drum velocity at all times. In fact, despite various claims to the contrary (Westheimer, 1954), it is possible in this way to elicit smooth tracking movements of the eyes to velocities matching drum velocities as high as $100^\circ/\text{sec}^{-1}$.

This however calls for considerable conscious effort and is difficult to maintain for any period of time even with quite low drum velocities. Instead, examination of recordings carried out under these conditions show that the eye movements fall into two types, those in which the eye velocity accurately matches that of the drum velocity and those in which the eye velocity is consistently inferior to that of the drum. In fact what appears to be happening is that despite conscious effort the nystagmic response repeatedly lapses from the "active" to the "passive" type response. In

the clinical situation therefore even though the patient may be instructed to follow the stripes of the drum it is in general the "passive" type which will be elicited by virtue of his inability to sustain the "active" response.

Now it is clearly apparent that the mechanism of this response must be quite different from that of "active" optokinetic nystagmus. Since the velocity of the eyes does not reach that of the drum there exists a velocity error between the two so that the images of the stripes continually traverse the retina. In consequence since the fovea is not dominantly involved as in the cases of "active" optokinetic nystagmus, the slow component of this type of nystagmus is not a true tracking movement, in which volition is an essential feature but rather it is the progression of the images of the stripes across the retina which appears to provoke the nystagmus. That this contribution from the peripheral retina is indeed a very potent stimulus may be judged from the fact that when foveal vision is absent as in patients with central scotomata the optokinetic response may be considerably enhanced (Hood, 1967). These observations have an important bearing upon

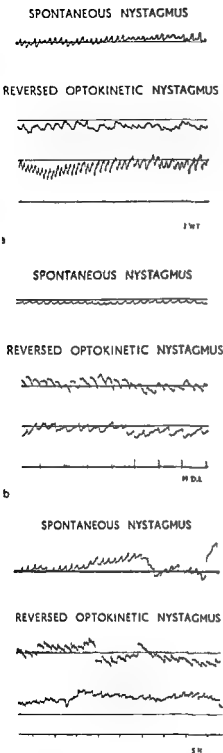


Fig 6 Spontaneous nystagmus and reversed optokinetic response in patients with (a) Miners nystagmus (b) Cerebellar encephalopathy (c) Multiple sclerosis

velocity is at times considerably slower than normal

As will be seen from the tracings of spontaneous nystagmus in each case although they are manifestly not vestibular in character neither are they truly pendular and in consequence such irregularities as exist will tend to persist in the course of their transformation by the optokinetic stimulus. Vagaries of form of this kind are of course to be expected in accordance with the hypothesis put forward, indeed their existence can be said to lend added support to it.

The second group of subjects three in all, had complete unilateral ophthalmoplegia. Tracings of the optokinetic responses of these subjects were obtained by means of simultaneous recordings from each eye, first with the normal eye covered and the paretic eye uncovered and secondly with the paretic eye covered and the normal eye uncovered. Although under neither of these conditions were we able to detect any movement of the paretic eye exposure of this eye to the optokinetic stimulus consistently induced a vigorous in direct response in the covered normal eye. Similar findings have previously been reported by Ohm (1926), Kornmüller (1931) and others.

Recordings from the normal eye under conditions of direct and indirect stimulation are

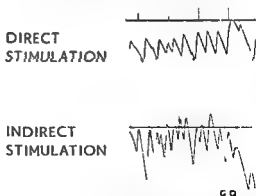


Fig 7 Optokinetic responses from subject with unilateral ophthalmoplegia. Stimulus $20^\circ/\text{sec}^2$. Direct stimulation—response from normal eye, paretic eye covered. Indirect stimulation—response from normal eye induced from stimulation of paretic eye, normal eye covered.

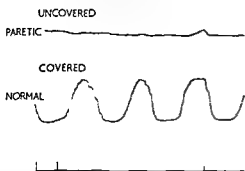


Fig 8 Following movements induced in normal eye of subject with unilateral ophthalmoplegia from stimulation of paretic eye, normal eye being covered. Note absence of movement in tracing from paretic eye

shown in Fig 7 and it will be seen that in this instance the indirectly induced response is enhanced in respect of both amplitude and frequency. However, although in all three subjects we never failed to elicit an indirectly induced nystagmus, enhancement was by no means a consistent finding in any subject. Nevertheless these findings do seem to provide ample confirmation of our contention that it is the passage of the images of the stripes across the peripheral retina which is the stimulus largely responsible for the optokinetic response. Furthermore as might be expected, the reported illusion of turning was always greater when derived from the paretic eye.

The tracings shown in Fig 8 are from one of the three patients but involve a different visual stimulus. Again, the normal eye was covered and the paretic eye uncovered. To the front of the paretic eye a target was moved smoothly from side to side over an arc subtending some 20 degrees.

The result as can be seen, is that despite the fact that no movement occurred in the paretic eye the moving stimulus gave rise to a simultaneous smooth pendular movement of the covered normal eye. Subsequent measurements have shown the extent of this movement to be 60 degrees considerably exceeding that of the moving target.

This finding is difficult to explain in terms of current notions and would appear to have significant implications in the theory under-

lying ocular following movements. It is conventionally held that the only situation in which smooth following movements of the eyes can be elicited is when the image of a moving target is fixed and held upon the fovea. To this end it is necessary to suppose that a variety of neural feedback mechanisms are involved aimed at maintaining clear foveal vision upon the moving target.

The findings presented in this paper, while they do not contravene these principles suggest that other mechanisms may be involved and in particular that the role of the peripheral retina warrants further consideration.

ZUSAMMENFASSUNG

Wie gezeigt werden konnte, bestehen bestimmte signifikante Unterschiede in dem Charakter der optokinetischen Reaktion, und zwar je nachdem ob die Versuchsperson ihren Blick, aktiv oder, passiv auf eine sich drehende Trommel festigt auf die schmale weisse Langsstreifen aufgebracht sind. Es ist daher zu vermuten dass den beiden Reaktionen unterschiedliche nervöse Mechanismen unterliegen. Die Bewegung des Bildes über die Netzhaut scheint der Reiz zu sein der die, passive Variante auslöst und weiterhin ist sie weitgehend die Ursache für die Illusionseindrücke bei Rotation der Versuchsperson. Den gleichen Mechanismus kann man sich auch als Erklärung des Phänomens des umgekehrten optokinetischen Nystagmus vorstellen. Das Auftreten dieses Phänomens sowohl bei Patienten mit angeborenem Nystagmus wie auch bei Patienten mit nicht angeborenem Nystagmus stützt diese Erklärung. Die Augenbewegungen die bei Patienten mit einseitiger Ophthalmoplegie durch Reiz des paretischen Auges im normalen Auge ausgelöst werden, sind eine weitere Stütze für die Auffassung dass das periphere Sehvermögen zur Kontrolle der normalen Augenbewegungen beiträgt.

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INTERACTION BETWEEN THE UTRICLES AND THE VERTICAL SEMICIRCULAR CANALS

V Bilateral Selective Sectioning of Anterior or Posterior Ampullar Nerves or Unilateral Selective Sectioning of the Two Vertical Ampullar Nerves followed by Tilting around their Bitemporal or Longitudinal Axis

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Abstract Selective bilateral sectioning of the anterior ampullar nerves was performed in 29 cats. This procedure resulted in a spontaneous nystagmus upwards. If the animals were tilted around their bitemporal axis rose downwards nystagmus was inhibited. Tilting nose upwards increased the nystagmus frequency. Selective bilateral sectioning of the posterior ampullar nerves caused a spontaneous nystagmus downwards. Tilting nose downwards increased the nystagmus whereas tilting in the opposite direction caused its inhibition. Selective unilateral sectioning of the anterior and posterior ampullar nerves was followed by a rotatory nystagmus which increased if the animals were tilted around their longitudinal axis towards the non-operated ear and which was totally inhibited if they were tilted in the opposite direction. The cause of this phenomenon is discussed.

traction of activity, depending on whether the ipsilateral or contralateral utricle is activated. Earlier investigations (Fluor & Mellström, 1970) have shown that electrical stimulation of certain areas on the utricular surface causes distinct vertical eye movements. Consequently, because there is every reason to suppose that cooperation exists also between the utricles and the vertical semicircular canals, we have found it a matter of great interest to explore this problem whose existence has hitherto remained unknown.

In a series of articles the present authors (1973 a, b, c) have established that the utricles and the horizontal semicircular canals cooperate very intimately. An increased input from the utricular area that causes horizontal eye movements, facilitates nystagmus released from the ipsilateral horizontal semicircular canal, but simultaneously inhibits nystagmus released from the contralateral horizontal canal. From this it has been concluded that the input from the utricle goes to the same oculomotor muscles as those receiving impulses for the slow phase of horizontal nystagmus. There is either a summation or a sub-

MATERIAL AND METHOD

Twenty nine cats, divided into three groups, were used for the experiments.

1 Selective bilateral sectioning of the anterior ampullar nerves, followed by tilting around the bitemporal axis of the cat (13 cases).

2 Selective bilateral sectioning of the posterior ampullar nerves, followed by tilting around the bitemporal axis of the cat (13 cases).

3 Selective unilateral sectioning of the two vertical ampullar nerves, followed by tilting

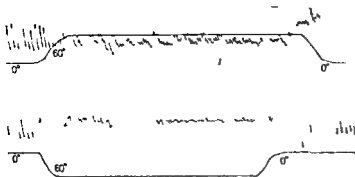


Fig 1 Consecutive curves from a cat after selective bilateral sectioning of the anterior ampullar nerves followed by tilting nose upwards and downwards. The upper curve shows electronystagmography and the lower curve the tilting of the table. Tilting nose upwards, curve upwards; tilting nose downwards, curve downwards.

around the longitudinal axis of the cat (3 cases).

The cats were first anesthetized with ether, by a so-called Ayer-system, which is employed in pediatric anesthesia. After completing the operation they were given Ketamine (Parke Davis), a non barbituratic anesthetic, characterized by pronounced analgetic properties but with minimal effects on the reticular formation in order to maintain a superficial level of anesthesia, without the animals moving or feeling pain, but with the reflex activity, including nystagmus remaining fully developed.

For sectioning of the anterior ampullar nerves the approach was the same as that for the horizontal ampullar nerve (Fluur & Siegborn, 1973). In order to expose the posterior ampullar nerve a retroauricular incision was made, and the bulla was opened. Immediately behind the round window a fenestration was

performed and the posterior ampullar nerve was exposed and cut. Great care must be taken not to damage the horizontal canal immediately above the ampulla, otherwise the cat developed horizontal nystagmus.

The eye movements were recorded partly by direct visual ocular inspection of the cat's eye, and partly by electronystagmography, with the electrodes above and below one eye, when recording vertical nystagmus. Because rotatory nystagmus cannot be recorded by any electrical method only visual inspection was applied in these experiments.

The animals were stimulated by tilting them 30 degrees or 60 degrees around their bi-temporal or longitudinal axis.

RESULTS

After bilateral, selective sectioning of the anterior ampullar nerves all the 13 cats showed vertical nystagmus upwards with 4–11 beats/10 sec. In all these cats tilting nose downwards resulted in inhibition of nystagmus, which, in 11 cases, was total (Fig 1). In 2 cats the frequency decreased from 5 to 2 beats/sec. If, on the other hand, the cats were tilted nose upwards, the spontaneous nystagmus increased in frequency by 1–17 beats/10 sec in 12 cats and remained more or less unchanged in one cat (Fig 1).

All 13 cats, where bilateral sectioning of the posterior ampullar nerve was performed, had vertical nystagmus downwards, with 8–21 beats/10 sec. If they were tilted nose down

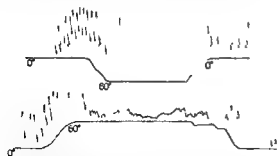


Fig 2 Consecutive curves from a cat after bilateral sectioning of the posterior ampullar nerves followed by tilting nose downwards and upwards. The upper curve shows electronystagmography and the lower curve the tilting of the table. Tilting nose downwards, curve downwards; tilting nose upwards, curve upwards.

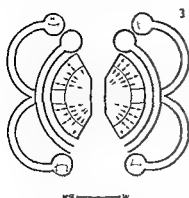


Fig 3 Schematic diagram of the utricles and vertical semicircular canals in resting position. The arrows indicate the orientation of the hair cells i.e., the direction in which they increase the discharge frequency

Fig 4 Schematic diagram of the utricles and vertical semicircular canals after bilateral selective sectioning of the anterior ampullar nerves and when the animal is in resting position. Arrow indication same as in

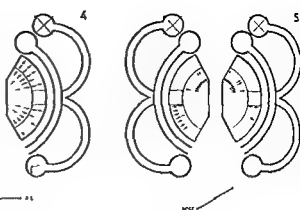
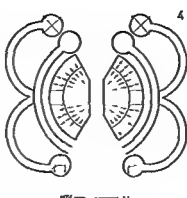


Fig 5 The number of arrows symbolizes discharge frequency

Fig 5 Schematic diagram of the utricles and vertical semicircular canals after bilateral selective sectioning of the anterior ampullar nerves and when the animal is tilted nose downwards. Arrow indication same as in Fig 3. The number of arrows symbolizes discharge frequency

wards, this caused definite increase in nystagmus frequency, by 6–27 beats/10 sec in 10 cases (Fig 2). In another 3 cats there was no substantial alteration in the nystagmus. If, instead, they were tilted nose upwards, nystagmus disappeared entirely in 11 cats, in 2 other cats nystagmus frequency decreased by about 6 beats/10 sec, but no total inhibition occurred (Fig 2).

In both these series it occurred of course, that one and the same cat could show both a decrease in nystagmus and total inhibition, depending on its degree of the wakefulness of the cat. But in general, reaction was always according to the principles involved. In some of the animals inhibition occurred immediately, in others it took about 5–10 sec.

Selective sectioning of the anterior and posterior ampullar nerves on the left side induced in the 3 cats on counterclockwise rotatory nystagmus (seen from the observer). If these cats were tilted around their longitudinal axis towards the non-operated ear, the nystagmus increased in frequency, when, however, they were tilted towards the operated ear the nystagmus disappeared completely.

DISCUSSION

The view expressed in earlier investigations (Fluur & Siegborn, 1973 *a, b, c*) concerning the interplay between the utricles and the horizontal semicircular canals, namely that the utricular influence on the horizontal eye movements is added to the slow phase of nystagmus, has proved to be fully valid also for the vertical semicircular canals regarding both vertical and rotatory nystagmus.

Earlier publications regarding the finer anatomical orientation of the sensory cells of the otolith organs (Lowenstein & Wersall, 1959) and the neurophysiological experiments concerning the utricular influence on the oculomotor activity (Fluur & Mellström, 1970) make it possible today, without theorizing, to explain the cause of the results obtained in the present experiments.

The electrical activity of the vertical semicircular canals and that of the 2 utricles are, in the resting position, completely balanced mutually (Fig 3). After selective sectioning of the anterior ampullar nerves the input from these semicircular canals disappears, and the

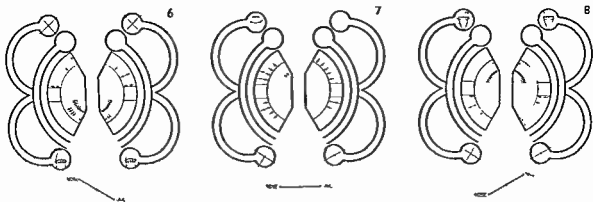


Fig 6 Schematic diagram of the utricles and vertical semicircular canals after bilateral selective sectioning of the anterior ampullar nerves and when the animal is tilted nose upwards. Arrow indication same as in Fig 3. The number of arrows symbolizes discharge frequency.

Fig 7 Schematic diagram of the utricles and vertical semicircular canals after bilateral selective sectioning of the posterior ampullar nerves and when the animal

is in resting position. Arrow indication same as in Fig 3. The number of arrows symbolizes discharge frequency.

Fig 8 Schematic diagram of the utricles and vertical semicircular canals after bilateral selective sectioning of the posterior ampullar nerves and when the animal is tilted downwards. Arrow indication same as in Fig 3. The number of arrows symbolizes discharge frequency.

cat experiences a vertical nystagmus upwards with the slow phase downwards (Fig 4). If the cat is now tilted nose downwards (Fig 5) total inhibition develops in those sensory cells on the utricular surface which induce eye deviations downwards at the same time as an increased input is being built up in those areas which cause elevation of the eyes. The outcome is a subtraction of input from or antagonism between the utricles and the posterior canals to the slow phase downwards. This inhibits the input to the oculomotor depressors and nystagmus disappears.

If on the other hand the cat is tilted nose upwards (Fig 6), the input increases in those utricular areas causing ocular depression and simultaneously the activity is inhibited in those areas causing elevation of the eyes. The outcome is a summation of input from or synergism between the utricles and the posterior canals to the slow phase downwards which increases the intensity of nystagmus upwards.

If however, the posterior ampullar nerves are cut the input from these semicircular canals disappears and the animal has vertical nystagmus downwards with the slow phase upwards (Fig 7). Tilting nose downwards

(Fig 8) inhibits those utricular areas causing depression of the eyes and the activity increases in those areas giving elevation. The outcome is an addition or synergism of activities from the anterior canals and the utricles to those oculomotor muscles giving elevation and this increases the nystagmus intensity downwards.

If the cat is tilted nose upwards (Fig 9) quite the contrary situation develops and the nystagmus downwards is completely suppressed.

If finally a selective unilateral sectioning of the anterior and posterior ampullar nerves is performed, the balance of activity is disturbed in favour of the contralateral vertical canals which induce a counterclockwise rotatory nystagmus with the slow phase beating towards the operated ear (upper eye pool) (Fig 10). If the cat is now tilted around the longitudinal axis towards the non-operated side (Fig 11) increased activity accumulates in the lateral part of the utricle which faces upwards and inhibition develops simultaneously in the medial parts. In the utricle facing downwards the situation is the contrary. This induces an increased input into those oculomotor muscles

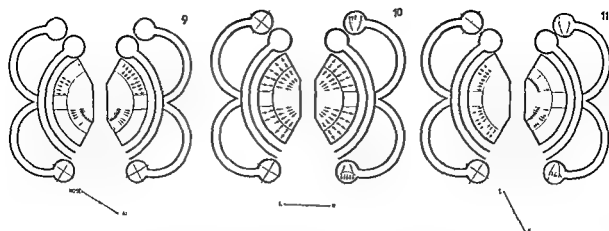


Fig 9 Schematic diagram of the utricles and vertical semicircular canals, after bilateral selective sectioning of the posterior ampullar nerves, and when the animal is tilted nose upwards. Arrow indication same as in Fig 3. The number of arrows symbolizes discharge frequency.

Fig 10 Schematic diagram of the utricles and vertical semicircular canals, after unilateral selective sectioning of the left anterior and posterior ampullar nerves,

and when the animal is in resting position. Arrow indication same as in Fig 3. The number of arrows symbolizes discharge frequency.

Fig 11 Schematic diagram of the utricles and horizontal semicircular canals, after unilateral selective sectioning of the left anterior and posterior ampullar nerves, and when the animal is tilted towards the right. Arrow indication same as in Fig 3. The number of arrows symbolizes discharge frequency.

which cause deviation of the upper eye pool in the direction of the operated ear. The result is again a summation or synergism of input from the intact, vertical canals and the utricles, which increases the intensity of nystagmus.

If, however, the animal is tilted in the other

direction, the result is entirely the opposite (Fig 12).

Consequently, it has been shown that the labyrinth is a very complicated computer, where every alteration in activity in any one of its separate sensory organs immediately in-

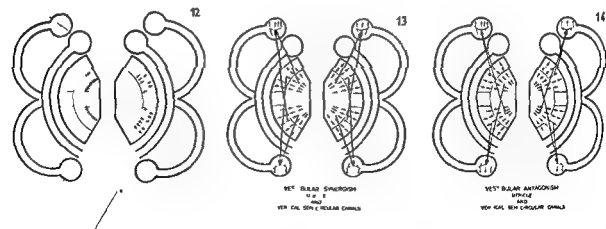


Fig 12 Schematic diagram of the utricles and vertical semicircular canals after unilateral selective sectioning of the left anterior and posterior ampullar nerves, and when the animal is tilted to the left. Arrow indication same as in Fig 3. The number of arrows indicates discharge frequency.

Fig 13 Schematic diagram showing vestibular synergism between the utricles and the vertical, semicircular canals.

Fig 14 Schematic diagram showing vestibular antagonism between the utricles and the vertical semicircular canals.

fluences the interplay between the other organs, so that an alteration occurs in the activity of the effector organs—in this case, the oculomotor muscles. Consequently, the vertical canals and the utricles also form one system of synergetically functioning sensory organs (Fig 13) and another system, which functions antagonistically (Fig 14)

ZUSAMMENFASSUNG

Bei 29 Katzen wurde eine bilaterale, selektive Abschnidung des vorderen vertikalen N. ampullaris vorgenommen. Dies ergab einen vertikalen Nystagmus nach oben. Eine Kippung in der bitemporalen Achse, mit der Nase nach unten, liess den Nystagmus verschwinden. Eine Kippung mit der Nase nach oben ergab eine Steigerung der Nystagmusgeschwindigkeit. Selektive, bilaterale Abschnidung des hinteren vertikalen N. ampullaris ergab einen vertikalen Nystagmus nach unten. Eine Kippung mit der Nase nach unten steigerte die Geschwindigkeit des Nystagmus. Eine Kippung in die andere Richtung inhibierte den Nystagmus. Wurde eine selektive, unilaterale Abschnidung des vorderen und hinteren N. ampullaris vorgenommen, so stellte sich ein rotatorischer Nystagmus ein, der sich steigerte, als die Tiere in der longitudinalen Achse nach dem nicht operierten Ohr hin gekippt wurden und der bei einer Kippung in die andere Richtung total verschwand. Die Gründe dafür wurden diskutiert.

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EYE MOVEMENTS AS A FUNCTION OF ACTIVE HEADTURNINGS

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Abstract Eye movements caused by turnings of the head present a very stable pattern with the eyes directed in the same direction as the headturnings. The initial deviation, the maximum deviation and the resting deviation of the eyes have been calculated as a function of the velocity of the head. This study is meant to form a basis for a future analysis of pathological cases in which other patterns of eye movements are found.

Eye movements caused by passive turnings of the head and body have been the topic of an extensive literature (Barany, 1906, Jongkees & Philipszoon, 1964, Aschan et al., 1956, Henriksson et al., 1972). Eye movements caused by passive turnings of the body in relation to the fixed head have been described in numerous papers (Barany, 1907, Magnus, 1924, Biemond, 1939 and 1940, Philipszoon & Bos, 1963, Bos & Philipszoon, 1963, Takemori & Suzuki, 1971).

Eye movements at active turnings of the head were studied by Gütlich (1962) who described the relations between the movements of the head when the subject wanted to fixate a target to the right or left in his field of vision. Gütlich described rapid deviation of the eyes in the direction of the active turning but made no quantitative estimations. Kurosawa (1965) used a linear accelerometer and electronystagmograph to determine the relation in time with the eye movements and head rotation and found different patterns in these relations. These patterns could be related to age but also to vestibular asymmetries such as preponderance.

Bartz (1966) studied with objective recordings the movement of the eyes and head in response to a visual target to the right and left. He studied

quantitatively the time relation between the head and eye movements. He found a rapid deviation of the eyes in the direction of the target largely preceding the movement of the head. After this rapid deviation of the eyes, a slow returning of the eyes was found to take place compensating the head movements. Some of this slow returning of the eyes was found to take place after the end of the head movements.

These studies of eye movements at active headturnings were all made in test situations in which the subject was actively engaged in looking towards some target located in the periphery of or outside his field of vision.

A different test situation is the one in which the test subject is actively turning his head without any special intention to direct his gaze for any kind of observing. A quantitative study of the involuntary eye movements appearing at such situations with active headturnings (concomitant eye movements) does not seem to have been the topic of any previous paper. This together with the fact that so many patients complaining of dizziness and vertigo claim that these difficulties arise or increase at active headturnings initiated the present study. The aim of this paper will therefore be to map out such concomitant patterns of eye movements at headturnings in normals.

METHOD

The turnings of the head were recorded by means of a potentiometer fixed on top of the

EYE MOVEMENTS AT HEAD TURNING

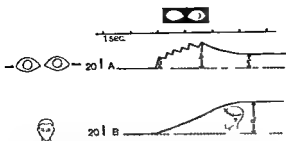


Fig 1 Normal pattern of eye movements at turning of the head (a) Initial deviation of the eyes (b) Maximum deviation of the eyes (c) Resting deviation of the eyes (d) Resting deviation of the head

head as previously described (Lundgren et al, 1969). The movements of the eyes were recorded with a conventional DC-recorder (Henriksson et al, 1972).

MATERIAL

Six normal subjects, aged 25–27, students of medicine without any previous vestibular complaints, were used as test subjects.

Procedure of the test

The test subjects were instructed with the help of a tape recorder. There was first given general information about the test and about what was expected from the test subject. The test subject was told to turn the head as much as possible without any discomfort.

The head was first turned to the right (one part turning), kept in this deviated position for three seconds and then turned back to mid-position. After three seconds in mid position a turning to the left took place, a pause for three seconds in this position and then again back to mid position. Thus, a complete turning was made up by four part turnings. Five such complete turnings to the right and left were done, four different velocities of the headturnings were used. On instruction from the tape recorder the subjects were told to turn their heads along with the counting from the recorder:

- 1 One turning in six seconds
- 2 One turning in four seconds
- 3 One turning in two seconds
- 4 One turning in one second

As four different turnings of the head were made (towards the right, back to mid position, towards the left and back to mid-position) at four different velocities, all together sixteen different kinds of headturnings were made. As each such turning was repeated five times, from each subject was achieved 80 recordings. As the tests were made both in light and darkness each subject presented 160 curves and as six subjects were used, the whole study was based upon 960 recordings of head- and eye movements.

The tests were all performed in the afternoon between 3 and 4 p.m.

Definition of parameters

For each of the sixteen different types of headturnings averages were calculated with respect to the following parameters:

- I Initial deviation of the eyes = amplitude of the first fast nystagmus phase (a in Fig 1)
- II Maximum deviation of the eyes = the maximum angular deviation of the eyes from their mid-position (b in Fig 1)
- III Total slow phase of all nystagmus beats – the sum of all slow phases achieved at one part turning of the head.

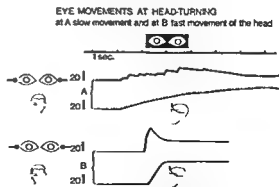
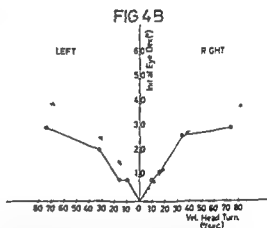
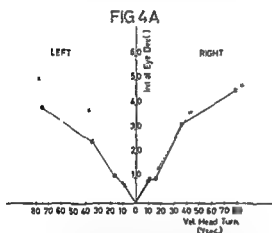
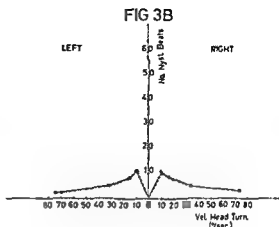
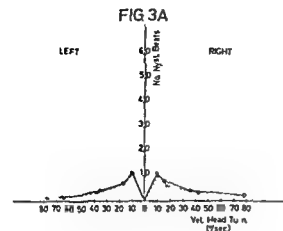


Fig 2 Simultaneously recorded position of the eyes and turning of the head. The two upper curves achieved at a slow turning of the head, two bottom pairs achieved at a rapid turning of the head.



Figs 3-8 Different parameters of eye movements in head turnings as a function of angular velocity of the head (A) Values measured in light (B) Values

measured in darkness ○—○ = Turnings of the head from mid position towards right and left ×—× = Returning of the head towards mid position

IV Average velocity of the eyes in the slow phases =

$$\frac{\text{Total amplitude of all slow phases}}{\text{Total time for slow phases}} \text{ } ^\circ/\text{sec}$$

V Resting deviation of the eyes—deviation of the eyes at the very end of the last slow phase (c in Fig 1)

VI The number of nystagmus beats during turning of the head

VII Angular velocity of the headturnings =

$$\frac{\text{Maximum deviation of the head}}{\text{Time for head turning}} \text{ } ^\circ/\text{sec}$$

A special interest was directed towards these above mentioned parameters as functions of the velocity of the head

RESULTS

- 1 All test subjects showed at each turning of the head a deviation of the eyes in the direction of the turning (Fig 1)
- 2 The fast headturnings resulted in only one fast phase of eye movements in the direction of turning and one slow phase in opposite direction (Fig 2) When the head was turned more slowly, the eyes performed a number of nystagmus beats in the direction of turning and always with a smaller number at high velocities of the head (Fig 2)
- 3 At the slow turnings of the head (one turning in six seconds) there was about ten nystagmus beats, while at rapid head movements the

FIG 5A

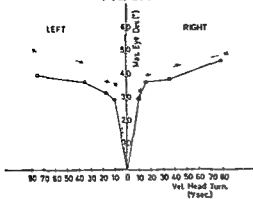


FIG 5B

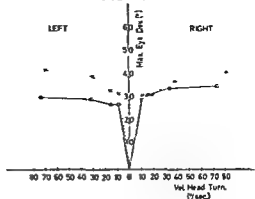


FIG 6A

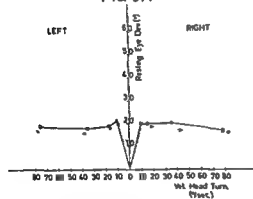
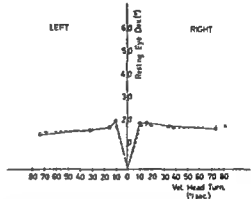


FIG 6B



eyes performed only one full nystagmus jerk (Fig 3 A and B)

- 4 The initial eye deviation increased with the velocity of the head. This deviation was almost a linear function of the velocity of the head up to 40°/sec, but seemed at higher velocities to increase more slowly. The initial deviation of the eyes was larger in light than in darkness (Fig 4A and B)
- 5 The maximum deviation of the eyes was always reached well before the end of the movement of the head and reached higher values at rapid turnings of the head. The maximum eye deviation was larger in light than in darkness (Fig 5A and B)
- 6 During and after the last part of headturning the eyes returned but did not fully reach their mid position. The resting deviation, still present when the eyes came to a rest, was

only slightly larger after slow than after high velocities of the head (Fig 6A and B)

- 7 The total slow phase amplitudes were found larger at slow turnings of the head than in rapid turnings. Further, total slow phase amplitudes were also larger in light than in darkness (Fig 7A and B)
- 8 The average slow phase velocity increased apparently as a linear function of movements of the head up to about 40°/sec. When the head was turned at higher velocities, the eyes seemed to lag behind the head. In darkness the velocity of the eyes do not even at slow velocity of the head correspond to the movement of the head (Fig 8A and B)

DISCUSSION

The results presented here indicate a very constant pattern. The eyes always deviated in the

FIG 7A

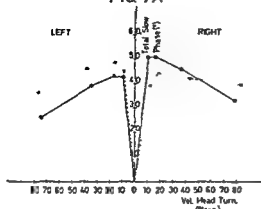


FIG 7B

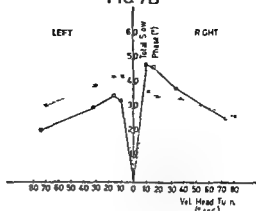


FIG 8A

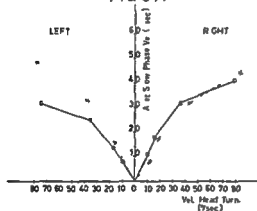
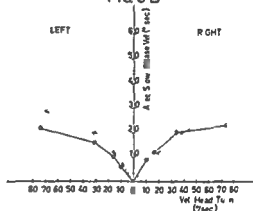


FIG 8B



direction of the turning of the head. An anticipated fixation of the eyes straight ahead was never found. It should also be pointed out that the test subjects only were instructed to turn your head.

Among possible factors responsible for the constant pattern of eye movements the following three mechanisms may be considered.

1 It has been shown that a torsion of the neck (also without turning of the head) causes a compensating deviation of the eyes and may also cause nystagmus (Philipszoon & Bos 1963, Takemori & Suzuki 1971). These eye movements are thought to be initiated by proprioceptive afferent impulses from neck muscles. It must therefore at least be discussed if the concomitant eye movements described here are to some extent released or at least facilitated by afferent activity in the neck muscles.

2 Another mechanism causing a deviation of the eyes in the direction of the headturning would be an anticompanying movement described by Melvill Jones (1964) and also later by Lundgren et al (1969) and by Goto et al (1968). Such a deviation seems to be provoked by intensive vestibular stimulation causing during the first part of the nystagmus reaction a displacement of the eyes always in the direction of the turning of the head. As however the concomitant eye movements show a quite normal pattern also in patients with no labyrinthine reactions the vestibular stimulation in the headturning could not possibly be considered responsible for the concomitant eye movements.

3 Impulses causing movements of the head may also be combined with parallel impulses reaching eyemotor nuclei causing meaningful deviation of the eyes in the same direction as

the head This linkage between quite different groups of muscles must be a common phenomenon in performance of frequently used complex movements and may be acquired in extra uterine life Its stability may be an expression of its meaningfulness It is interesting to mention in this connection that in newborn children such linkage is not yet developed In these very young children the eyes will deviate opposite the direction of the movements of the head (Doll's phenomenon) This deviation opposite to the movements of the head anyhow takes place at passive turnings of the head (Bickerstaff, 1963) The importance of the meaningfulness of the concomitant eye movements may be stressed by the fact that in blind persons there are less or no such concomitant eye movements

Irrespective of what the mechanism may be for this concomitant eye movements, a thorough knowledge of these eye movements in normal conditions must form a basis for an understanding of different patterns in pathological conditions

ZUSAMMENFASSUNG

Durch Kopfdrehungen verursachte Augenbewegungen in Richtung dieser Drehungen folgen einem sehr stabilen Schema Die Initial-, Maximal- und Ruhewerte für die Deviation der Augen sind als Funktion der Winkelgeschwindigkeit des Kopfes berechnet worden Die vorliegende Arbeit bildet den Ausgangspunkt für kommende Studien pathologischer Fälle, bei denen abweichende Arten der Augenbewegungen festgestellt werden können

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THE LOCALIZATION OF VESTIBULAR EFFERENT NEURONS IN THE KITTEN WITH HORSE RADISH PEROXIDASE

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Abstract The location of efferent vestibular neurons in the brainstem of newborn kittens was determined by means of horseradish peroxidase used as a tracer of retrograde protein transport. Two to three milligrams of horseradish peroxidase were injected into the vestibule of 8 kittens. After survival for 24 hours, the animals were fixed by perfusion and the brainstems sectioned. Stains for peroxidase demonstrated the labelled neurons lateral to the abducens nucleus bilaterally.

An efferent component of the vestibular nerve was first demonstrated in 1958 (Rasmussen & Gacek) and 1960 (Gacek) with axonal techniques following lesions in the *stria medialis* of the cat. Since then the efferent vestibular pathway has also been demonstrated with histochemical techniques utilizing the property of acetylcholinesterase localization in efferent nerves and terminals (Gacek et al., 1965, Gacek, 1967). Furthermore, the terminals of the efferent pathway have been described in detail with the electron microscope (Smith & Rasmussen, 1967). Over the past 15 years more than 100 lesions in the vestibular nuclei and cerebellum have been produced and the resulting degeneration in the vestibular nerve branches studied in order to determine the precise location of the origin of the vestibular efferent pathway. Utilizing this technique the cerebellum as well as the superior

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vestibular nucleus, medial vestibular nucleus and dorsal part of the lateral vestibular nucleus have been ruled out as a possible source of the efferent vestibular fibers. The probable location of the vestibular efferent neurons has been postulated in the vicinity of the ventral division of the lateral vestibular nucleus or possibly the reticular formation (Gacek, 1967). No indication of a contralateral origin to the bundle has been found by this author.

The traditional method of determining the cells of origin of a neural pathway is to elicit the retrograde cell reaction following transection of the cell's axon. Brodal's (1940) modification of this technique has been utilized in young kittens after unilateral labyrinthectomy but no unequivocal cell changes could be found that would justify location of the vestibular efferent neurons (Gacek, 1964). It is extremely difficult to identify chromatolysis and displacement of the nucleus in very small neurons. All available information on the size of the axons of the efferent neurons indicates that these are small sized neurons. Rossi & Cortesina (1962) did state on the basis of cell changes and cell loss following vestibular nerve section in the guinea pig that they could demonstrate the origin of the vestibular efferent bundles. They found two bundles, one arising from the lateral vestibular nucleus and a more ventral bundle which arose from a

nucleus called the 'nucleus interpositus' This small nucleus was located between the lateral vestibular nucleus and the descending trigeminal root However, because of the equivocal nature of the cell changes, this origin has not been generally accepted

Recent techniques have shown that exogenous proteins which have been presented to the neuromuscular junction of motor nerves such as the hypoglossal nerve (Kristensson et al, 1971, Sjostrand, 1969) or other motor nerves (Zacks & Saito, 1969), will be taken up by the axons and be carried in retrograde fashion to the cell body over a period of 10-24 hours Protein tracers that can be used to demonstrate this retrograde transport are Evans Blue albumin and horseradish peroxidase The tracer can then be demonstrated with staining techniques (Graham & Karnovsky, 1966) in the cytoplasm of the cell bodies located within the central nervous system This property appears to be age dependent and is more demonstrable in the newborn animal (Kristensson & Olsson, 1971)

In the present study newborn kittens were used to demonstrate the location of the cells of the vestibular efferent system in the brain stem after injection of horseradish peroxidase into the vestibule of the inner ear Following a survival time of 24 hours, the animals were sacrificed by perfusion, the brainstems sectioned and stained according to the method of Graham & Karnovsky (1966) The cells of the vestibular efferent system were selectively demonstrated with horseradish peroxidase in an area between the abducens nucleus and the ventral division of the lateral vestibular nucleus bilaterally

MATERIAL AND METHODS

Eight newborn kittens of age 1 day or less were used as the experimental animal The animals were anesthetized with intraperitoneal Nembutal (0.5 cc/kg) and the vestibule of the inner ear exposed by two approaches 1) In 4 animals an atticotomy approach was used

to approach the oval window after making a skin incision posterior and medial to the right auricle After removal of the incus, the mucoid substance which fills the middle ear space was aspirated and the stapes superstructure removed leaving only the footplate of the stapes in the oval window The footplate was elevated carefully and perilymphatic fluid aspirated Then, with a syringe and a fine gauge needle, 20-30 μ l of solution containing 2-3 mg of horseradish peroxidase in Ringer's solution was injected into the vestibule and the footplate replaced The middle ear was then filled with a free muscle graft and the incision closed with sutures 2) In three kittens a ventral approach through the bulla was utilized to approach the vestibule After removal of the cartilaginous covering of the bulla and evacuation of the mucoid contents of the bullar space, the round window niche and membrane were identified and the round window membrane carefully perforated The injecting needle was then directed in a superior direction so that the needle point perforated the most basal end of the cochlea and penetrated the vestibule from a ventral direction The 20-30 μ l of horseradish peroxidase solution was then injected into the vestibule and gelfoam was then placed in the round window niche This approach was also utilized because the horseradish peroxidase, when injected through the oval window, was difficult to contain within the vestibule and a certain amount of overflow into the middle ear space had resulted In the ventral approach the reflux of horseradish peroxidase was minimal 3) In one animal the atticotomy approach was utilized and the middle ear exposed The incus was removed and mucoid substance aspirated as in the other animals No opening into the vestibule was produced but the same quantity of horseradish peroxidase was instilled into the middle ear space so as to contact the mucosa and the wound was closed

All 8 animals operated on were allowed to survive for 24 hours Then they were again anesthetized with Nembutal and intracardiac



Fig 1 Photomicrograph (magnification 1200 \times) of vestibular efferent neurons labelled with horseradish peroxidase. The HRP granules are visible in the cyto-

plasm of 3 neurons. Compare to unlabelled neuron (arrow).

perfusion was utilized to sacrifice the animals. Following a saline flush with the catheter placed in the left ventricle and an exit incision made in the right auricle, the fixative of 4% formaldehyde and 5% glutaraldehyde in a 0.1 M phosphate buffer with a pH of 7.2 was used. The calvarium was removed and the entire head immersed for 24–36 hours in the fixative before removing the brainstem and cerebellum from the skull. Further fixation was allowed in the fixative of 4% formaldehyde and 5% glutaraldehyde and then frozen sections were cut at 25 microns. Sections were then incubated for 10–15 minutes at room temperature in a saturated solution of 3,3'-diaminobenzidine. The sections were washed in 3 changes of distilled water and then mounted on slides using gelatin adhesive. After dehydration to absolute alcohol for 10 minutes, the sections were counterstained in

0.1% cresylviolet and differentiated through 95% alcohol. After dehydration and clearing, the sections were covered and ready for examination. Complete sets of serial sections were obtained through the brainstem from rostral to the superior vestibular nucleus to the caudal portion of the brainstem. These sections were then carefully evaluated and cells with the rusty red horseradish peroxidase granules (Fig 1) were located on an outline drawing of the section.

RESULTS

The chart in Table I summarizes the three groups of animals described in the method section. The 4 animals in which the horseradish peroxidase was injected by an oval window approach to the vestibule all demonstrated uptake of the tracer in small cells which were

Table I Chart summarizing the results in experimental and control animals following injection of horseradish peroxidase (HRP)

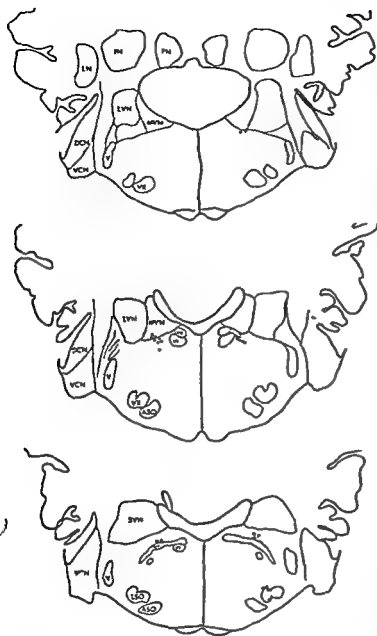
Animal no	Site of injection (HRP)	Uptake in vestibular efferents	Location in brainstem	Number of cells labelled	
				Ipsilat	Contralat
K 1-020273	Vestibule (O W)	Very good (3 +)	Small cells lateral to VI nucleus	145	58
K 2-020273	Vestibule (O W)	Good (2 +)	Same as above	72	32
K 1-022673	Vestibule (O W)	Good (2 +)	Same as above	70	23
K-1-031273	Vestibule (O W)	Weak (1 +)	Same as above	7	3
K 1-022673	Vestibule (R W)	None	Diffuse in surface cells of brainstem	—	—
K-2-031273	Vestibule (R W)	Strong (4 +)	Small cells lat to VI nucleus	174	151
K 1-040273	Vestibule (R W) bilat	None	Diffuse in surface cells of brainstem	—	—
K 3-031273	Middle ear	None	—	—	—

grouped lateral to the abducens nucleus and medial ventral to the lateral vestibular nucleus. This localization of cells with the protein tracer occurred bilaterally in all cases. However, the intensity of uptake in these cells varied between animals. In animal K 1 020273, there were 145 cells ipsilateral to the injection side and 58 on the contralateral side. Although the 3 remaining animals demonstrated a lesser intensity of uptake and therefore fewer cells that were countable, it was evident that the number of cells ipsilaterally was greater than contralaterally in all three cases.

The second group of 3 animals, in which the injection was performed through the hook portion of the basal turn of the cochlea into the vestibule, gave a different experience. This method of approach to the vestibule was selected in an attempt to maintain a better concentration of horseradish peroxidase within the vestibule. In the group of animals where the tracer was injected through the oval window after elevating the stapes footplate, considerable peroxidase overflowed into the middle ear space and was difficult to contain within the vestibule even with soft tissue plugging of the oval window niche. In two animals (K-1-022673 and K-1-040273) the peroxidase was not taken up within any cells in the ves-

tibular nuclei but became diffusely located in the surface cells of the ependyma of the brainstem indicating that the injection had been into the cerebrospinal fluid space. It became clear that with this approach the peroxidase was sometimes injected into the internal auditory canal and the modiolus. However, in one animal (K-2-031273) the injection into the vestibule was successful and the horseradish peroxidase was contained entirely within the vestibule with no leakage into the cerebrospinal fluid space. In this animal the uptake was stronger than in any of the animals in the first group. This was evidenced by a greater density of tracer within the cells and in a greater number of cells contralaterally than were identified by the oval window approach. Cell counts in this animal indicated there were 174 cells ipsilaterally as compared with 151 contralaterally. The location of labelled cells in this animal is shown in Fig 2 where representative levels of the vestibular nuclei are shown. The rostro-caudal extent of the labelled cells approximated that of the abducens nucleus.

Finally, a control animal (K-3-031273), where the horseradish peroxidase was injected into the middle ear revealed absence of labelled cells in the brainstem and cerebellum. The

*List of abbreviations used in figures*

- LVN = lateral vestibular nucleus.
 MVN = medial vestibular nucleus.
 SVN = superior vestibular nucleus.
 DCN = dorsal cochlear nucleus.
 VCN = ventral cochlear nucleus.
 ASO = accessory superior olivary nucleus.
 LSO = lateral superior olivary nucleus.
 V = descending trigeminal root.
 VI = abducens nucleus.
 VII = facial nerve.
 RB = restiform body.
 VR = vestibular root.
 LN = lateral (dentate) nucleus.
 PN = interposed nucleus.
 FN = fastigial nucleus.

Fig 2 Outline drawings of the brain stem of K. 2-031273 showing the location of labelled neurons (black dots) following injection of horseradish peroxidase into the right vestibule

possibility of other efferent neurons being labelled with peroxidase was ruled out with this control.

Two groups of efferents that could conceivably be involved are 1) the motor nerves to the middle ear muscles and 2) autonomic preganglionic efferent neurons of the seventh and ninth cranial nerves. The stapedius and tensor tympani muscles are innervated by branches of the seventh and fifth cranial

nerves. The nuclei of these nerves are located in the brainstem at the caudal and rostral levels of the vestibular nuclei. No labelled cells were seen in the facial nucleus or the motor trigeminal nucleus.

The autonomic efferents travel via (a) the pars intermedia of the seventh cranial nerve through the chorda tympani nerve to the submaxillary ganglion and (b) the tympanic branch of the ninth cranial nerve through the

tympanic plexus and lesser superficial petrosal nerve to the otic ganglion. The cells of origin of these autonomic nerves are located in the superior and inferior salivatory nuclei respectively within the brainstem. While their nerve terminals are far removed from the middle ear space, it was considered possible that the peroxidase could penetrate the intratympanic portions of the axons. The absence of tracer in the brainstem of the control, however, provided evidence against this possibility.

DISCUSSION

While the phenomenon of anterograde axonal transport has been known for some time (Lasek, 1968, Ochs et al., 1967, Taylor & Weiss, 1965), the concept of retrograde transport of proteins from the nerve ending into the cell body has only been recently demonstrated (Kristensson et al., 1971, Kristensson & Olsson, 1971, Zacks & Saito, 1969). Several investigators have demonstrated that protein tracers such as Evans Blue albumin and horseradish peroxidase can be identified in motor neurons in the central nervous system after presentation of these protein tracers to their motor endplates. This has been clearly demonstrated in the hypoglossal and vagus nerves as well as the motor nerves to limb muscles such as the gastrocnemius muscle. These studies have been carried out in newborn animals because in the newborn animal the perineurium is open-ended peripherally (Zacks & Saito, 1969) as contrasted to adult animals where the perineurium constitutes a barrier for proteins to diffuse into endoneurium and contact the axon (Kristensson & Olsson, 1971). It is therefore possible to present an exogenous protein tracer such as horseradish peroxidase at the axon terminal, and then after a period of time, at least 10 hours and preferably 24 hours, the protein tracer can be identified in the cytoplasm of the neurons whose axons have been in contact with the protein tracer. An important factor in determining the uptake of protein in the axons is the concentration of the

tracer around the terminals. This was substantiated in the present study where the greater concentration of peroxidase injected by the round window approach in K-2-031273 produced a stronger uptake and more cells labelled than in the animals injected via the oval window technique where the peroxidase leaked out of the vestibule. The present study showed that, after injection of horseradish peroxidase into the vestibule, 5 out of 7 animals had consistent localization of horseradish peroxidase in small cells located just lateral to the abducens nucleus and ventro-medial to the ventral division of the lateral vestibular nucleus (Table I). While the amount of uptake in these cells via the oval window injection of the peroxidase was variable, this approach was technically easier and less likely to contaminate the cerebro-spinal fluid space. On the other hand, while better concentration could be attained through a round window approach, in 2 of the 3 animals where this approach was used, the peroxidase entered the cerebro-spinal fluid space and was not useful for localization of the efferent neurons to the inner ear. An indication that concentration of the horseradish peroxidase in the perilymphatic space of the vestibule was important was clearly brought out by the amount of uptake of peroxidase in the labelled cells of animal K-2-031273 as well as the number of cells so labelled. The increase in labelled cells in this animal as compared to animal K-2-031273 has primarily in the number of contralateral efferent neurons that were demonstrated. These neurons are located at a considerable distance from the side of injection and therefore would require a greater concentration of peroxidase to be labelled in the same time period. The total number of efferent neurons in one labyrinth as determined in this study agrees with the number of efferent neurons as revealed by degeneration techniques. This number is estimated at 200-300 (Kristensson, 1971).

The possibility that the efferent neurons are located in the brainstem lateral to the

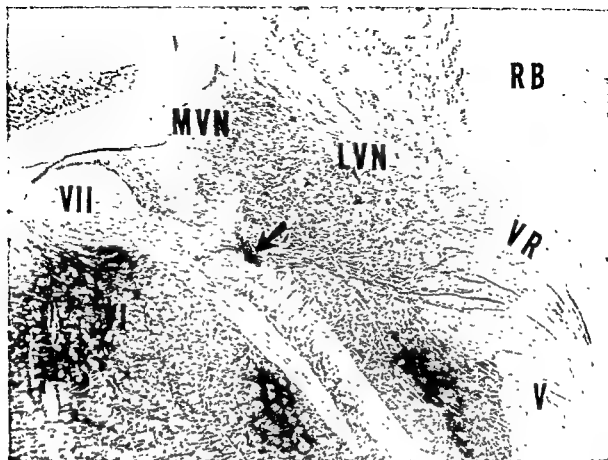


Fig 3 Photomicrograph of normal adult cat brainstem demonstrating high acetylcholinesterase activity in a localized area ventro-medial to the lateral ves-

tibular nucleus (LVN) and projecting fibers into the vestibular root (VR).

longed to autonomic portions of the seventh and ninth cranial nerves, or to the innervation of the stapedius or tensor tympani muscles was ruled out by the injection of the horseradish peroxidase into the middle ear of kitten K-3-032173. No uptake was seen in any cells of the brainstem of this animal.

An additional indication that the labelled neurons are those of vestibular efferents is found in animal K-2-031273 (Fig 2) where some labelled cells were seen in the dorsal perimeter of the lateral superior olivary segment. This is precisely where the neurons giving rise to the uncrossed olivo-cochlear bundle have been located by other methods. Undoubtedly some horseradish peroxidase travelled up the scala vestibuli and down into the

upper portions of the scala tympani where diffusion through the osseous spiral lamina into the perilymphatic space of the organ of Corti provided the route for uptake of exogenous protein by efferent cochlear fibers. With a longer period of time and greater concentration of peroxidase in scala tympani more olivocochlear efferent neurons can be labelled with this technique (Warr, 1973).

The present location of efferent vestibular neurons is further supported by the acetylcholinesterase localization technique where an area of high activity has been noted medial and ventral to the lateral vestibular nucleus (Fig 3). Acetylcholinesterase positive fibers emanating from this location can be seen travelling into the dorsal portion of the vestibular

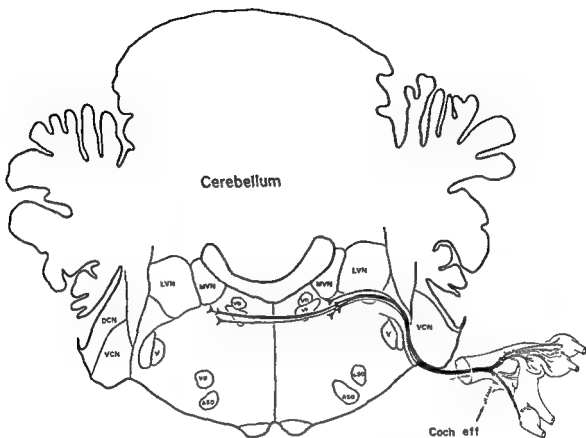


Fig 4 Drawing of the origin, course and termination of the efferent vestibular pathway

root where they become associated with the outgoing olivo-cochlear fibers. Furthermore, lesions producing axonal degeneration which have been used to demonstrate the peripheral portions of the bundle in the past have localized the most probable site of efferent vestibular neurons to an area near the ventral portion of the lateral vestibular nucleus or the reticular formation. Degeneration experiments such as these, however, have failed to indicate bilaterality to the origin of the vestibular efferents. Possible reasons for this failure to demonstrate a contralateral bundle are 1) difficulty in staining fine degenerating efferent fibers in the peripheral nerve segments with silver or myelin techniques and 2) the midline transections of the olivo-cochlear

bundle were not deep enough to cut the axons of the contralateral efferent fibers. This would indicate that the axons of the contralateral vestibular efferent neurons cross the midline at a deeper level than olivo-cochlear fibers.

The drawing in figure 4 depicts the origin, course and termination of the vestibular efferent pathway and its relationships to the efferent cochlear bundle. The present location of the vestibular efferent neuron differs from the "nucleus interpositus" described by Rossi & Cortesina (1962). He described an entirely ipsilateral origin for the vestibular efferent system, one was located in the lateral vestibular nucleus giving rise to the "dorsal direct vestibular efferent bundle" and the second was the "nucleus interpositus" which was located

between the lateral vestibular nucleus and the descending trigeminal root and gave rise to the "ventral direct vestibular efferent bundle". Acceptance of this origin was not universal because of the previously mentioned difficulty in determining unequivocal retrograde changes in small neurons where scanty cytoplasm is normally seen and where eccentric nuclei may be present in the normal animal Carpenter (1960) on the basis of widespread retrograde cell changes following labyrinthectomy ascribed a bilateral origin in the cerebellar nuclei and several vestibular nuclei. Again equivocal cell changes and the disproportionately large number of neurons involved, prevented acceptance of these studies as indicative of the origin of vestibular efferent fibers.

A consideration of the location of the efferent neurons with regard to the incoming afferent innervation from the labyrinth is significant. At their location, ventral to the medial and lateral vestibular nuclei, these neurons are located in an area where the descending branches of first order neurons from the cristae and maculae converge. The opportunity is present, therefore, for direct termination of the afferent neurons from all vestibular sense organs onto the efferents. This is not surprising when one considers that the axons of the efferent fibers travel out the vestibular nerve and disperse evenly to the hair cells in all of the sense organs.

A number of physiological experiments on the efferent vestibular system have been conducted most notably those by Schmidt (1963), those of Gleissner & Henriksson (1964), and finally of Sala (1965). Of these, only that of Sala has attempted to stimulate the efferent vestibular system by placing the stimulating electrode within the vestibular complex. His stimulating electrode was placed ventrally in the lateral vestibular nucleus (p. 16 in Sala, 1965) and appears very near the location of the vestibular efferent neurons as described in this study. The inhibitory effect on vestibular nerve activity was probably mediated via the contralateral efferent component. In

such a case, however, it may be noted that only half of the vestibular efferent influence to one labyrinth was being stimulated.

With the precise location of the efferent vestibular neurons the opportunity now exists for more accurate physiological evaluation of this system. A major reason for conducting such morphological studies is to aid in the design and interpretation of neurophysiological investigation.

ZUSAMMENFASSUNG

Die Lage der efferenten Neuronen im Stammhirn neugeborener Katzen wurde über den retrograden Transport von Proteinen mittels Meerrettich Peroxydase bestimmt. Zwei bis drei Milligramm Meerrettich Peroxydase wurden bei 8 Katzen ins Vestibulum injiziert. Nach 24 Stunden wurden die Tiere fixiert und ihre Stammhirne wurden geschnitten. Die Färbung der Peroxydase zeigte die markierten Neuronen lateral auf beiden Seiten des Nucleus abducens an.

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REINNERVATION AFTER RESECTION OF THE FACIAL NERVE

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Abstract After radical parotidectomy for carcinoma carried out in a five month-old child spontaneous return of the function of the facial musculature developed gradually during the second year after the intervention. Electromyographic studies showed that reinnervation developed mainly from the homolateral (involved) side even though the contralateral (uninvolved) side evidently also participated in the reinnervation of the mouth sphincter. Responses could be evoked from the superficial layers of the subcutis in the midface. The authors therefore assume that in the case described the unexpected reinnervation was effected by dual innervation of the buccinator. It is likely that in some persons this muscle is also activated by the motor fibres of the trigeminus.

In recent years much interest has been paid to the physiology of the facial musculature due to the successful replacement of the missing segments of the facial nerve by nerve grafts and to the so far unresolved problem of the spontaneous return of the function of the facial muscles in patients whose seventh cranial nerve had been resected and no effort made to reconstruct its anatomical continuity by surgical intervention. Spontaneous reinnervation was observed to occur more frequently after resection of the whole extratemporal portion than after loss of an endotemporal portion of the facial nerve.

This unusual phenomenon is the subject of controversy and remains an unresolved problem. The regenerating axons can reach

the paralysed facial muscles in some uncommon manner, either via the homolateral or the contralateral seventh cranial nerve or via the fifth cranial nerve.

To our knowledge only about 30 such cases have, so far, been reported in the literature. Aberrant reinnervation was described for the first time by Martin & Helsper (1957 and 1960) though it had already been observed earlier by other authors. Martin et al. observed this phenomenon in eight cases in connection with total radical parotidectomy whereas Conley (1963) in five persons and Trajaborg & Siemssen (1972) in four patients in whom the same operation had been performed. Other authors reported only single cases of spontaneous reinnervation after loss of the extratemporal portion of the seventh cranial nerve. Silverstein et al. (1967) encountered this phenomenon in one patient with a destroyed tympanic portion of the facial nerve by middle ear teratoma.

The findings described in this paper might contribute to the problem of spontaneous reinnervation whose importance increases both from the theoretical and the clinical point of view.

CASE REPORT

A five month-old girl was referred to our clinic with an enlarging mass in the right parotid gland which had reached the size of a plum within a period of 3 months. The tumour was

Presented on the occasion of the Congressus Oncologicus et Tumores Infantium held in Karlovy Vary on September 23rd 1972.



Fig 1 Total facial nerve paralysis caused by a tumour

hard and painful and led to a spontaneous development of total facial nerve paralysis (Fig 1). Sialography disclosed destructive changes in the parotid gland. Fine needle aspiration biopsy established the malignant nature of the mass (Fig 2). On May 29th 1970, total radical parotidectomy was performed. The sternocleidomastoid and masseter muscles and the cervical lymph nodes with the internal jugular vein were resected. The intervention was completed by tarsorrhaphy. Histopathology showed an immature cancer. The bioptic specimen from the lymph nodes was negative. Post-operatively, the right parotid region was irradiated with Cobalt⁶⁰ teletherapy (total dose 3 300 R).

The first voluntary motion of the mouth angle appeared 16 months after surgery. Supported by rehabilitation measures, the motion of the facial muscles gradually extended over the whole face. Two years post operatively, the tumour did not recur and the face

appeared symmetrical when at rest. The functional weakness was apparent, however, on emotional activity especially in the involved lower part of the face (Fig 3).

The electromyographic (EMG) studies of the buccinator revealed a simple or intermediate response during voluntary motion (Fig 4a). The reaction of the orbicularis oris muscle was recorded on stimulation with a bipolar surface electrode. The latency time measured on stimulation of the facial nerve on the affected side was 58 msec (Fig 4b) whereas that from the normal side was 10 msec (Fig 4c). The response from the depressor oris inferior muscle had a latency time of 5 msec, and from the zygomatic muscle it did not appear at all. No response was obtained on stimulation of the plexus cervicalis. When the bipolar needle stimulation electrode was inserted superficially in mid face, the response was recorded from the orbicularis oris muscle. On deeper insertion in front of the external auditory canal, and toward the foramen stylomastoideum, no response could be obtained. The evaluation of the results was difficult because the examination was painful and the child restless.

DISCUSSION

The spontaneous and unexpected return of motion following surgical section or resection at a distance of some centimetres distal to the ganglion geniculi is made possible only by motor fibres belonging either to the seventh or the fifth cranial nerve. The voluntary motor impulses cannot be transmitted to the paretic muscles via sensory or vegetative nerves (Wright, 1967). The axoplasmatic flow from the central stump of the facial nerve disappears in the scarring area. It is unlikely that outgrowth sprouts of the main nerve trunk could persist in such a milieu and penetrate up to the paralysed facial muscles.

Our interpretation of the spontaneous re-innervation is based on the above mentioned

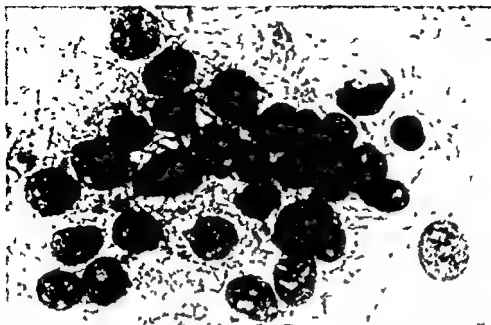


Fig 2 Smear of the parotid gland tumour (Pap penheim ME) $\times 1200$ Group of tumour cells with

veiled grey bluish plasma Anisomorphism anisochromy and pathological nuclei

grounds The central communications between the motor nuclei of the facial and trigeminal nerve are well known (Braus 1960) The parietic facial musculature can receive the impulses via three possible anatomical pathways through the motor nucleus of the facial nerve from the cortex

Contralateral reinnervation from the facial nerve of the uninvolved side

It is well known to the neurologist that the unaffected side can partly substitute the function of the involved side The mandibular branches of the facial nerve on both sides have overlapping areas of innervation This observation was made by us while carrying out a dissection study of the peripheral division of the extratemporal portion of the seventh cranial nerve in several persons (Černý et al 1970) The contralateral reinnervation from the non parietic side brings about only the motion around the mouth angle This phenomenon might not always be conditioned by overgrowth of the axons inside the sphincter oris muscle According to present knowledge, the maximum participation of the un

involved side in the anomalous innervation is 20% (Passerini 1968) A more important role is ascribed to contralateral reinnervation by Fisch (1968)

This can be verified either by procaine infiltration at the circumference of the mouth angle on the uninvolved side or by comparison of the latency time after simultaneous stim



Fig 3 Ch \square 2 years after operation

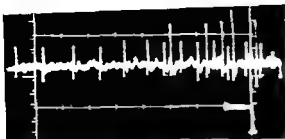


Fig 4 (a) Contraction activity during crying from the *m. buccinatorius* dx. Potentials of two motor units with frequency of 20–40 cycles/sec. Calibration 100 μ V/50 msec.

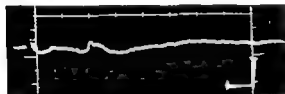


Fig 4 (b) Evoked potential from *m. orbicularis oris* dx. Stimulation near the tragus of the contralateral side with bipolar surface electrode. Calibration 150 μ V/5 msec.

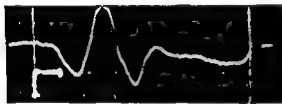


Fig 4 (c) Evoked potential from *m. orbicularis oris* dx. Stimulation near the tragus on the ipsilateral side with bipolar surface electrode. Calibration 150 μ V/2 msec.

ulation from the paralytic and the normal unaffected side during EMG studies. For ethical reasons, we did not use nerve bloc with anesthetics but the electromyographic stimulation tests. Thus proof could be provided that the mouth sphincter was partly innervated from the contralateral side.

Reinnervation from the facial nerve of the affected side

A dominant role in the spontaneous return of the function of the paretic facial muscles is attributed to the involved seventh cranial nerve by Conley (1963). Unlike other inves-

tigators, he did not succeed in inhibiting all the renewed functions of the facial muscles by anesthesia of the maxillary and mandibular branches of the fifth nerve. The results obtained showed a rarefaction in muscle activity on potentials at voluntary activity, which occurs in neurogenic atrophy or at reinnervation. According to Conley, the phenomenon of spontaneous reinnervation might be due to unknown aberrant connections via the branches of the facial nerve. He also takes into consideration the geniculate ganglion with its two petrosal nerves and the extra-cranial ganglia which develop from the seventh cranial nerve (Gasser, 1967). He concludes that under abnormal conditions the impulses might reach the paretic muscles along these hardly imaginative anatomical pathways.

(a) The ganglion oticum, the junction of the fibres of the fifth and seventh cranial nerve, may represent an important point of homolateral spontaneous reinnervation of the mimetic muscles. The motor fibres of the facial nerve reach the ganglion oticum either via the lesser petrosal nerve through a strong communication *cum plexu tympanico* (Viliger, 1943), or via the communicating ramus between the lesser and greater petrosal nerve on the upper pyramidal surface (Sobotta & Figge, 1963).

(b) The composition of the neural elements of the ganglion sphenopalatinum and of the greater petrosal nerve is not in accord with the requirements of aberrant innervation. This nerve contains only rarely insignificant amounts of motor fibres (Gardner et al., 1963).

(c) The severed central stump of the main trunk of the seventh cranial nerve might also serve as a source of homolateral reinnervation. Binns (1972) expressed this viewpoint on the basis of the results obtained from experiments carried out on cats. McCoy & Boyle (1971) are of the same opinion. This hypothesis is, however, hardly acceptable as far as man is concerned because it contradicts the

theoretical knowledge and the clinical experiences derived in neurosurgery

The better innervation from the affected side was demonstrated in our patient. It is difficult, however, to determine whether the restored function of the facial muscles is attributable to the seventh cranial nerve.

Reinnervation from the fifth cranial nerve

In aberrant innervation, only the motor branches of the portio minor of the trigeminal nerve may be of use. This portion innervating the masticatory muscles is characterized by a variable ramification and its branches by a variable course. Silverstein et al (1967) and Mundnich (1967) assume on the basis of clinical estimations that anastomoses between the motor component of the fifth and the seventh cranial nerve exist in ca 5–15% of all humans. However, a reliable proof in that respect could not be provided as yet. These rami communicantes probably arise during embryonal life, since the trigeminal and facial nerves are related, as far as the development is concerned, to the same branchial origin (Gasser, 1967).

Thus many authors have motivated the observed spontaneous reinnervation. They argue that the assumed loss of aberrant innervation, which has been restored after previously carried out surgical intervention, does not always take place, when extended radical reoperation for recurrent parotid cancer is performed. After the local nerve block of the maxillary and mandibular branches of the fifth cranial nerve or after alcohol injections, indicated in cases of accidentally coinciding trigeminal neuralgia, only a transient cessation of face motion is noticed. A spontaneous reinnervation may be effected by the following pathways of the trigeminal nerve:

(a) Via the masseteric nerve which supplies both the similarly named muscle and the deep layer of the temporal muscle whilst the superficial layer is innervated from the facial nerve.

(b) Via a thick anastomosis between the buccal branch of the fifth nerve and the buc-

cal branch of the seventh cranial nerve (Braus, 1960). This sensory branch penetrating the buccinator is joined by a small bundle of motor fibres (Hill, 1946) which also seem to contribute to its innervation.

(c) Via the large communicating rami between the auriculo-temporal and facial nerves. The presence of these anastomoses, which are 1–3 in number, and the connections with the otic ganglion have recently been reported by Baumel et al (1971). They contain, besides the secretomotor twigs and vasodilator fibres destined for the glandular vessels also motor fibres for myoepithelial cells and smooth muscles of the duct system of the parotid gland.

CONCLUSION

In our patient, spontaneous reinnervation developed from the ipsilateral side. We could not establish how and along which anatomical pathway it was effected, whether the anastomoses lying inside or around the mentioned muscles influenced it or whether the outgrowth of axons from the masticatory muscles penetrated into the denervated mimetic muscles. The evoked potentials were recorded from the superficial layers of the subcutis in the middle of the face where the fibres of the buccal branches of the fifth and seventh cranial nerves are situated, both of them lying above the buccinator.

We assume that in some persons this muscle is also supplied from the trigeminal nerve. Dual innervation of the musculus buccinatorius or connections between motor fibres from buccal branches of both cranial nerves may play an important role in spontaneous reinnervation of the facial muscles.

Obviously, the boundary-line of the development and the innervation of the mimetic and masticatory muscles is not so sharp as described in textbooks on embryology and in anatomical atlases. The authors are of the opinion that, besides all the other pathways, the motor portion of the trigeminal nerve

might play its role in spontaneous reinnervation. Further details and specific studies concerning the variability of innervation of this region which arises in prenatal life, are still required.

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ZUSAMMENFASSUNG

Nach radikaler Parotidektomie wegen Carcinoma die bei einem 5 Monate alten Säugling durchgeführt wurde kam es im Laufe des zweiten Jahres nach dem Eingriff zu spontaner Reinnervation der Gesichtsmuskulatur. Elektromyographisch wurde festgestellt, dass die Innervation des Mundspinkters vorwiegend von der homolateralen (betroffenen) Seite ausging, obwohl sich daran auch die kontralaterale (gesunde) Seite beteiligte. Es ist uns gelungen, aus den oberflächenschichten der Unterhaut im mittleren Gesichtsfeld in der Region des Buccinator Potentiale hervorzurufen. Die Autoren schliessen daraus, dass im gegebenen Fall die unerwartete Funktionsrückkehr durch zweierlei Innervation dieses Muskels zustande kam. Sie nehmen an, dass dieser Muskel bei manchen Personen auch durch die motorischen Nervenfasern des Trigemini versorgt wird.

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COMPRESSION AND ISCHAEMIA OF THE FACIAL NERVE

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Abstract Two factors possibly involved in ischaemic facial paralysis in man were investigated in the cat compression by injecting fluid between the nerve fibres and ischaemia due to circulatory arrest. The nervous function was assessed by observation of nerve action potentials, the lesions by histological examinations. About 3 1/2 hours of suprasystolic compression was critical. The effect is attributed to occlusion of the arterial circulation. Infrasytolic compression had less influence, infradiastolic compression no influence. Histological examinations demonstrated disruptions of the nerve fluid between the nerve fibres and vascular stasis. An increased vulnerability of the nerve to ischaemia is attributed to the presence of oedema between the nerve fibres. If the theory about ischaemic paralysis in man is correct, a treatment interrupting the vicious circle of compression and ischaemia should be instituted in severe cases within the first day(s) in order to prevent denervation.

The prevailing theory about the mechanism of acute idiopathic peripheral facial paralysis (Bell's palsy, rheumatic facial paralysis, facial paralysis *a frigore*) is that of combined primary and secondary ischaemia. This theory, advanced by Kettel (1947), Hilger (1949), Sullivan & Smith (1950), is inspired *inter alia* by the experimental work of Denny Brown & Brenner (1944 a, b). It can be summarized as follows (Fig. 1): a local arteriolar constriction (the primary ischaemia) provokes an increase in permeability of the arteriolar wall due to hypoxaemia, the ensuing transudate then compresses the facial nerve fibres, an impairment of the venous and lymphatic return of the circulation causes more oedema compressing the nerve fibres, and ischaemia of secondary nature. A vicious circle is

created in this way. Theoretically, the nerve fibres could be saved by any therapeutic action which breaks the vicious circle before they have degenerated. Unfortunately, the cause of the arteriolar constriction is not known, it may be allergic, toxic, endocrine or psychic (Miehke, 1960). An unequivocal explanation may never be found, since various factors are capable of triggering the same pathogenetic mechanism (Zulch, 1970). The term "ischaemic paralysis" for this condition was introduced by Kettel (1954). Since the ischaemia is generally accepted as the triggering factor in this paralysis (Miehke, 1960) that name will be used here.

Our study was limited to the vertical (mastoid) part of the facial nerve which is in our opinion the most vulnerable part of the nerve for several reasons. In the human facial canal it is the only segment where the nerve fibres are collected into a single fascicle (Sunderland & Cossar, 1953) and, at the same time, are surrounded by a thick perineurium of 25 to 35 μ formed by concentric layers of dense collagenous connective tissue with few elastic fibres (James, 1961). Nerve fibres in monofascicular nerves are more susceptible to compression than fibres in multifascicular ones (Sunderland, 1968), and the hyperkalaemic depolarization of the nerve fibres by ischaemia may be additive in closely packed adjacent nerve fibres (Fox & Kenmore, 1967). Moreover, in a retrospective study of about 300 de

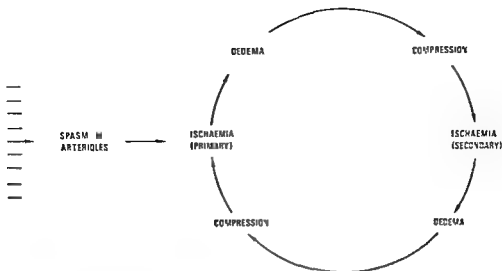


Fig. 1 Schematic representation of the pathogenesis in ischaemic facial paralysis

compressions of the vertical part of the nerve in cases of ischaemic paralysis we have seen not a single case of recurrent paralysis of a nerve that had been operated upon. On the contrary, among 440 patients with ischaemic paralysis 19 non-operated patients had a second attack of facial paralysis, 12 of them within 10 years after the first attack, and 2 non-operated patients even had 3 attacks at the same side of the face within 26 years (Devriese & Pelz, 1969). On the other hand we have seen a second attack of ischaemic paralysis after decompression of the nerve of the other side in a few instances. Since decompression of the vertical part seems to exclude a recurrence on the same side, one may surmise that the pathology is localized chiefly in that segment of the nerve.

Cats were chosen for our experiments since in the vertical part of the facial canal their facial nerve is also monofascicular and surrounded by a thick perineurium. Two factors involved in the ischaemic theory, viz. the compression by oedema and the ischaemia were studied inside the facial canal. For a more extensive description of the experiments the reader is referred to an earlier publication (Devriese, 1972b).

METHODS

The right zygomatico-orbital branch of the facial nerve of the cat was stimulated antidromically by a constant-current stimulus with a bipolar electrode. The monophasic nerve action potentials were recorded by means of a glass electrode from the facial nerve that was cut in the middle ear just distal to the geniculate ganglion. The compression of the nerve fibres was realized by injecting Locke's solution for cats (Eyzaguirre, 1960) into the facial nerve within the sheath in the vertical segment of the facial canal. This method, originally used by Jain & Sharma (1964) in the rabbit, was modified substantially. The pressure system for "infusion compression" is depicted in Fig. 2. The 25 gauge hypodermic needle was occluded at the tip and polished in such a way as to locate the tip more centrally. Small holes of 0.3 mm were drilled in the lateral walls as near as possible to the tip. Any sharp edge was carefully polished away. The pressure system was filled with Locke's solution corresponding to the extracellular fluid. Heparin (0.6%) was added to prevent clogging of the needle. The hypodermic needle was inserted under the

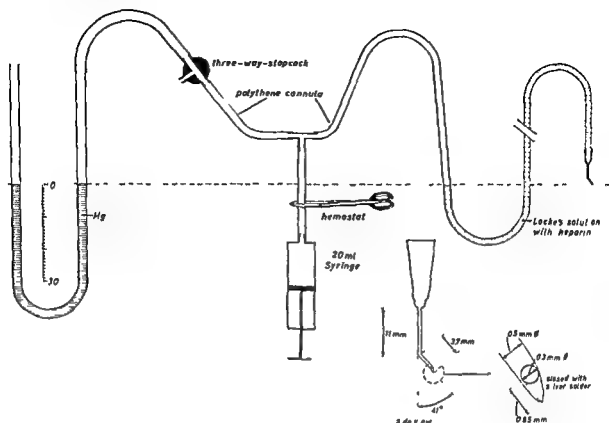


Fig 2 Pressure system for infusion compression of the facial nerve. In the right lower corner a schematic

description of the modified hypodermic needle is given.

operating microscope (magnification $10\times$) via the stylomastoid foramen into the facial nerve. This was done under continuous inspection of the action potential on the oscilloscope. The needle was fixed on a micromanipulator, the mercury manometer was adjusted to the same level as both the needle and the facial nerve.

The blood pressure of the cat was measured by means of a catheter introduced into the abdominal aorta. In 12 cats the blood pressure values ranged 130–160 mmHg/systolic and 90–120 mmHg/diastolic.

In suprasystolic compression the pressure inside the compression system was kept between 150 and 320 mmHg, in infrastolic compression below the systolic blood pressure but above the diastolic blood pressure, and in infradiastolic compression under the diastolic blood pressure.

The facial nerve with the needle inside it

was prepared for histological investigation. The fixation was initiated *in vivo* with the pressure still on the nerve by dripping SUSAFixative (Romeis, 1968) on the stylomastoid foramen for about 15 min. After decapitation of the animal the temporal bone was removed for further fixation in SUSAFixative, dehydration in an alcohol series, embedding in Rollwax 1 (Lamb, London) and decalcification in 5% nitric acid in a saturated solution of picric acid (70% ethanol).

Ischaemia was obtained by arrest of the blood circulation at the end of an experiment. A saturated solution of potassium chloride (3 mol) was injected intravenously.

RESULTS

1 Suprasystolic compression

Electrophysiology. Fig 3 gives a three-dimensional display of the compound nerve action

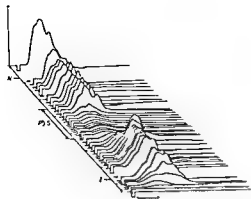


Fig 3 Three-dimensional display of nerve action potentials before during and after 105 min of reversible suprasystolic infusion compression. The first curve of the experiment is displayed in the left upper corner the last one in the right lower. Each curve represents 500 averaged action potentials. Calibrations 250 μ V (vertical bar) and 1 msec (horizontal bar). The time (oblique axis) is indicated in hours. *N* indicates the insertion of the needle. *P>S* supra systolic compression. *I*, ischemia. Experiment 41. SD (stimulus duration) 150 μ sec. SI (stimulus intensity) 300 μ A. MA (maximal amplitude in the experiment) 189 μ V. RT (total recording time of the experiment) 7 h 27 min.

potentials during the experiment. The nerve was compressed for 105 min. The compression was followed by an almost complete disappearance of nerve activity. As soon as the pressure was released the action potential reappeared. Finally, the action potential became about the same as before the compression although small differences in recovery time in certain fibres groups were still evident 2 hours after compression. This experiment ended with recording of action potentials after arrest of the circulation (ischaemia). Fig 4 gives a survey of the action potential under the influence of compression of 210 minutes' duration. After release of pressure, the amplitude of the nerve action potential remained negligible during the remainder of the observation period.

Table I and Fig 5 give a survey of 9 experiments during which infusion compression of increasing duration was performed. The amplitude of the action potential in percent of the amplitude before infusion compression

(ordinate) is plotted against the time from the onset of compression (abscissa). After release of the pressure the amplitudes regained their original values in 6 of the experiments. Generally, the longer the compression lasted, the slower was the return of nervous activity. Although the amplitude of the action potential recovered in experiment 48 after 180 min of compression there was a change in shape and a delay of the action potential. In experiments 60, 62 and 67 where the compression lasted for 210 or 245 min a rapid return of action potentials could no longer be observed. There is a distinct difference in the effect of release after short and after long periods of compression. Clogging of the needle was excluded after removal of the needle at the end of the experiment.

Histology. In Plate 1 the site in the nerve where the tip of the needle was located is shown. Around the insertion site the nerve fibres are packed together. In other parts of the nerve, oedema and disruption can be seen. At a higher magnification (Plate 2), in a section likewise treated with the Mallory Azan connective tissue technique, the dilatations of

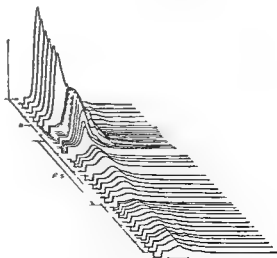


Fig 4 Two-hundred and ten minutes of suprasystolic infusion compression. Expt 60. SD 280 μ sec. SI 600 μ A. MA 374 μ V. RT 8 h 25 min. The nerve action potential remained negligible after relief of the pressure.

the endoneural connective tissue between the nerve fibres and vascular stasis can be observed

2 *Infrasystolic, infradiastolic, and mixed compressions*

An example is given in Fig 6. The blood pressure of the cat, the pressure in the infusion compression system, and the parameters of the action potential during the same experiment are given in Fig 7. At the beginning of the experiment, infradiastolic compression of 70 mmHg for 50 min did not influence the action potential. Infrasytolic compression of 125 mmHg was then attempted for 3 hours. After 75 min, however, the systolic blood pressure of the cat decreased and the compression exceeded the systolic blood pressure. The amplitude reacted immediately. Fifty five minutes later the blood pressure increased again and the effect of compression decreased. After release of pressure the action potential returned to the precompression level. Finally, the nerve was submitted to 42 min of suprasystolic compression and to ischaemia.

3 *Ischaemia*

Ischaemia was studied in 12 cats: in 2 of the control group, in 1 after a decompression operation and in 9 after a reversible pressure block of the nerve (the nerve block was judged reversible when the action potential recovered in amplitude and configuration after release of the pressure). In Fig 8 the maximal amplitude of the action potential (in μV) is plotted against the time after arrest of the blood circulation (in minutes).

The ischaemia in experiments 31 and 89 was preceded by a control experiment: in experiment 26 by a successful decompression of the nerve, and in the other 9 by a reversible compression. It is seen from the figure that the influence of ischaemia in experiments 26, 31 and 89 differed distinctly from that in the other nine. In the former case the nerve resisted the process of ischaemia for a relatively

Table 1 *Suprasystolic infusion compression of the facial nerve for more than 40 minutes in 9 cats*
See also Fig 5

Exp. no	Action potential after insertion of needle (percentage of initial value)	Amplitude before applying pressure (μV)	Duration of pressure (minutes)
72	44	96	42
38	64	170	58
55	32	100	76
41	50	108	105
36	50	219	122
48	90	373	180
62	67	604	210
60	70	190	210
67	100	516	245

long time, the action potential decreased to 50% after 17 to 45 min, then it broke down rapidly. In the latter case the action potential decreased more rapidly after the onset of ischaemia, falling within 15 min to 50%, thereafter it fell more slowly.

DISCUSSION

The difference between the normal action potential and that observed after a pressure block must be due either to inactivity of nerve fibres or to slower conduction in the depressed area or to both (Gasser & Erlanger, 1929). Due to the short distance of conduction the nerve fibres groups could not be differentiated in the action potential. The fact that the action potential never disappeared completely during compression could be attributed to action potentials generated in the intact part of the nerve fibres distal to the nerve block. The function of the nerve can thus be influenced by injecting fluid into it. It should further be stressed that this was done in the normal anatomical situation of the facial nerve inside the facial canal.

As demonstrated in experiment 72 (Figs 6 and 7) the nerve action potential reacted sharply to small variations in blood pressure around the systolic blood pressure. This proves that the actual pressure inside the

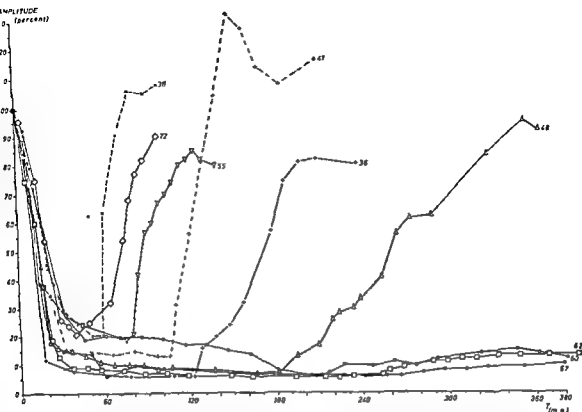


Fig 5 Amplitudes of nerve action potentials during and after suprasystolic infusion compression of the facial nerve Ordinate amplitude in percent of the amplitude before compression Abscissa time in

minutes from the onset of compression The figure at the end of each curve indicates the number of the experiment See also Table I

nerve corresponded to the pressure in the infusion compression system and that the effect of infusion compression is clearly related to the blood pressure of the animal Since the most distinct effects were seen during suprasystolic compression, the conclusion is reached that the arterial circulation of the nerve must play a determining part The initial changes caused by localized pressure are probably due to the associated ischaemia even if subsequent more pronounced structural changes are the results of anatomical deformation by the compression (Sunderland, 1968)

About $3\frac{1}{2}$ hours of suprasystolic compression appeared to be the critical duration of compression compatible with early functional recovery Infrasystolic compression above the

diastolic blood pressure had little influence on the nerve action potential, as soon as it exceeded the systolic blood pressure the influence of suprasystolic compression became distinct (Fig 7) The effect of infrasystolic compression may be provoked by occlusion of the venous return with preservation of the arterial supply

Infradiastolic compression did not influence the nerve function In our opinion this is due to persistence of the circulation

The action potential decreased faster after ischaemia than after compression This can be explained by the fact that infusion compression required about 40 min to build up a nerve block by accumulation of a sufficient amount of fluid between the nerve fibres It should also be noted that the pressure block

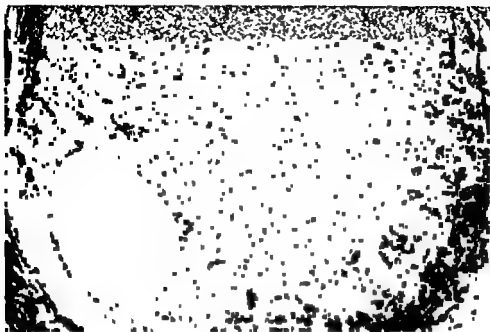


Plate 1 Transverse action of the facial nerve submitted to infusion compression for one hour. Around the site where the tip of the needle was localized

(left) the nerve fibres are packed together. Dilatations of the endoneurial space are shown in the other parts of the nerve. Cat 84 Mallory-Azan $\times 180$

was limited to a small part of the facial nerve in the facial canal, whereas ischaemia involved the total length of the nerve under study. In vivo, compression cannot be realized without interference with the circulation in the nerve. The results clearly indicate a difference between the influence of ischaemia on intact nerves and on nerves previously subjected to infusion compression. The only difference between the two groups of nerves was that in the second group Locke's solution with heparin was injected into the nerve. Although the nerve action potential recovered after the short period of compression these nerves were more vulnerable to ischaemia. This could be explained by the fact that fluid between the nerve fibres prevented diffusion of oxygen from the surrounding tissue towards the nerve.

It follows that if oedema is indeed present between the nerve fibres in cases of ischaemic paralysis, the present experimental evidence demonstrates the importance of maintaining an adequate blood supply to the nerve during

its surgical decompression (Miehlke, 1960, Kettel & Jongkees, 1963).

It has been demonstrated experimentally that decompression of the vertical part of the normal facial nerve can be performed without measurable damage to nerve function if performed with the utmost care (Devriese, 1972a). Interference with the circulation of an oedematous nerve is probably more hazardous.

Although the hypothesis of combined primary and secondary ischaemia in ischaemic paralysis in man is accepted, several factors still have to be proven. The swollen appearance of the nerve after slitting the nerve sheath has been regarded as evidence of pathological swelling under pressure but may occur also when the perineurium of a normal nerve is opened (Sunderland & Cossar, 1953).

According to Jongkees (1965), however, the abrupt ending of the bulging of the decompressed oedematous nerve, in contrast to the non-bulging part, is the typical feature of ischaemic paralysis. The localization of the

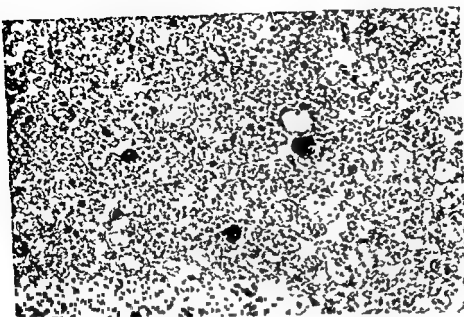


Plate 2 Higher magnification of a facial nerve submitted to compression for 1 hour. The endoneurial space is dilated. Note the filling of small venules

and capillaries (stasis) and the dilated arteriole. Cat 79 Mallory-Azan $\times 500$

swelling may also vary, it may involve the tympanic portion of the nerve (Williams, 1959) or even the part of the facial nerve proximal to the geniculate ganglion (Fisch & Esslen, 1970). Histological examinations of the facial nerve or the chorda tympani in cases of ischaemic paralysis have been re-

ported by Jongkees (1954), Kettel (1959), Miehke (1960), McGovern (1970), and others. Degenerative signs in the myelin sheaths (swelling) and less frequently in the axons, were found. No apparent signs of inflammation such as infiltration by leucocytes were reported. Examinations of the epineurium of the chorda tympani (Jongkees, 1954, Sade et al., 1965, Sade, 1972) was negative: no oedema, no inflammatory changes or blood vessel anomalies could be discerned. The oedema, however, could have disappeared before histological examination was performed (Kettel, 1947). No definite conclusion about the pathology of ischaemic paralysis can be reached without studies of normal temporal bones (Anson et al., 1970), more autopsy studies using advanced histological techniques (Fowler, 1963), and careful examination of the patients to either diagnose or exclude other diseases. Finally it should be noted that only very few attempts have been made to measure the pressure inside the nerve (Jongkees, 1968), these allowing no definite conclusion.

If the theory of combined primary and sec-

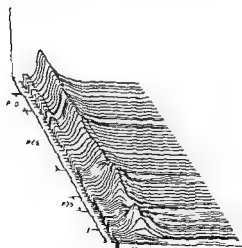


Fig 6 Three periods of compression of the facial nerve. Infradiastolic compression ($P < D$) for 50 min, infrasytolic ($P < S$) for 180 min and suprasystolic ($P > S$) for 42 min. I. Ischaemia Exp 72 SD 140 μ sec EI 600 μ A MA 97 μ V R⁺ P 1.2 V

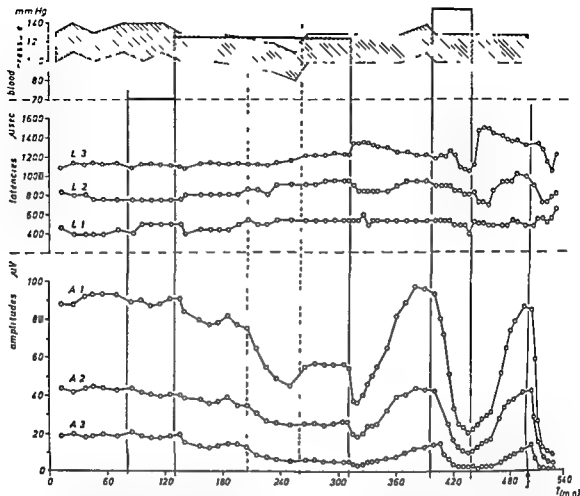


Fig 7 Infradiastolic infrasytolic and suprasystolic infusion compression of the facial nerve. Same experiment as in Fig 3. Six parameters of the nerve action potential are also represented. L3 latency time

to the centre of gravity. L2 latency time is the maximum of the derivative. L1 latency time to the onset. A1 maximum amplitude. A2 approximate surface. A3 amplitude of the derivative.

ondary ischaemia starting the vicious circle of ischaemia and compression is correct, the sudden ischaemic paralysis in man can be explained by occlusion of the arterial circulation to the nerve. In the cat a critical duration of nerve block was reached after about 3½ hours of interruption of the arterial circulation. Under the influence of infrasytolic (supradiastolic) compression the nerve function decreased more slowly and would eventually have disappeared completely. Unfortunately, this could not be checked since the experiments could not be continued for more than about 400 minutes.

Our results support the statement of Bun-

nell (1952) that the only possible time for prophylaxis or prevention of denervation is during the first three hours if the blockade of the circulation is complete. If it is partial it would presumably take a few days longer. Naumann et al (1968) also suggested on electrical grounds that the most critical period for the nerve is the first 24 hours. The best results of treatment can then be reached by interrupting the vicious circle of compression and ischaemia in severe cases at the earliest possible moment the first day or days. This could explain the results recently reported after early treatment with corticosteroids (Tave-

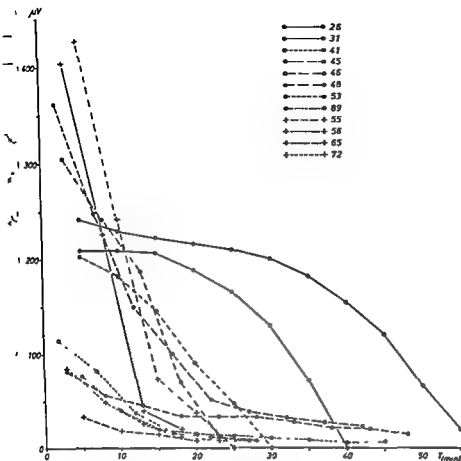


Fig. 8 Influence of ischaemia on the maximal nerve action potential in 12 cats. The maximal amplitude of the action potential (in μV) is plotted against

the duration of ischaemia (in minutes). Inset: the number of the experiment.

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Figures 2 to 8 and the Plates are published with the kind permission of the Royal Dutch Academy of Sciences.

ZUSAMMENFASSUNG

Zwei Faktoren welche möglicherweise auf die ischämische (Bell'sche) Fazialislähmung beim Menschen

bezogen werden können, wurden bei der Katze untersucht. Kompression durch Einspritzung von Flüssigkeit zwischen die Nervenfasern, und Ischämie durch Blockierung der Blutzirkulation. Die Nervenfunktion wurde mittels Beobachtung der Nervenaktionspotentiale geschätzt, und die Läsionen wurden histologisch untersucht. Ungefähr $3\frac{1}{2}$ Stunden suprasystolische Kompression waren kritisch, was der Abschliessung der arteriellen Zirkulation zuzuschreiben ist. Infrasytostische Kompression hatte weniger und infradiastolische Kompression keinen Einfluss. Bei histologischen Untersuchungen wurden Einrisse im Nerv, Flüssigkeit zwischen den Nervenfasern und vaskuläre Stauungen gefunden. Ödem zwischen den Nervenfasern verursachte eine grössere Anfälligkeit der Nervenfasern für Ischämie. Ist die Lähmungstheorie durch Ischämie beim Menschen korrekt, so musste man versuchen, den Circulus vitiosus von Kompression und Ischämie innerhalb der ersten Tagen durch zu brechen, um Denervation vorzubeugen.

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PERIPHERAL FACIAL PALSY IN SUB-ARCTIC NORWAY

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Abstract The results of a prospective five-year study of peripheral facial palsy in sub-arctic Norway are reported. The idiopathic form accounted for 78.6% of the cases and the incidence for the total population was 10.8/100 000/year. This figure is not significantly higher than those reported from several other, more temperate regions. The incidence of Bell's palsy was low under 20 years of age, increased markedly in the third decade of life and showed no tendency to falling-off in the elderly. No significant seasonal variation was found, nor was there any correlation between age group and season of the year.

Idiopathic peripheral facial palsy is a common and distressing clinical problem which has been accorded increased attention in the literature of recent years. In spite of intensive clinical and experimental investigation there is still uncertainty as to the incidence, aetiology and preferred mode of treatment. Considerable agreement has been reached in the management of certain aetiological groups of peripheral facial palsy, in particular the traumatic, otogenic and herpes zoster paralyses, but the situation with respect to the idiopathic form, or Bell's palsy, which is by far the commonest, is still undecided.

It is widely accepted that the idiopathic form of peripheral facial paralysis is caused by an ischaemia of the intra temporal course of the nerve as a result of a raised intraneural pressure secondary to microvascular pathology (Jongkees, 1954; Kettel, 1959), and this theory constitutes the theoretical justification for the operation of decompression. Exposure to cold has been frequently cited

as a possible cause of reflex ischaemia resulting from dysfunction of the autonomic nervous system (Kettel, 1959; Miehke, 1960; Zulch, 1970), and this factor has been proposed as a possible explanation for the differing epidemiology of idiopathic facial palsy in different areas of the world (Martin, 1952).

The literature contains comparatively few studies of the incidence of idiopathic facial palsy, and those studies which have been performed present differing results and conclusions. The present investigation was therefore instituted to establish the incidence of peripheral facial palsy in a part of the world with a stable population and characterized by rather severe climatic conditions.

MATERIAL AND METHODS

Our hospital serves the northernmost part of Norway, which lies north of the arctic circle and has a population of approximately 177 000. Statistics show that this total is comparatively stable, with an annual population swing of not more than 0.3% in the five-year period 1967-71. In 1966 medical colleagues throughout the region were contacted by letter and requested to refer all patients with peripheral facial palsy for investigation and treatment as soon as the condition was diagnosed. Almost all referrals were admitted to the department of otolaryngology.

A fixed battery of investigations was per-

Table I Aetiology of peripheral facial palsy in the five year period 1967 to 1971 (n=173)

	1967	1968	1969	1970	1971	Total	%
Temporal bone fracture	1	4	2	4	3	14	8.1
Operative trauma			1	1		2	1.15
Otitis media, acute		1			1	2	1.15
Herpes zoster cephalicus	1	1	1		1	4	2.3
Parotid tumour					1	1	0.6
Melkersson-Rosenthal syndrome		2	1	2	1	6	3.5
Pregnancy	1		2 ^a	2	3	8	4.6
Bell's palsy	12	24	33	39	28	136	78.6
Total	15	32	40	48	38	173	100.0

^a One patient with polyneuritis gravidarum

formed and a complete neurological examination made. The former included a general physical and otolaryngological examination, roentgenological examination of the temporal bones, electrical gustometry and the Schurmer test, audiological and vestibular work ups and fasting blood sugar and herpes zoster antibody estimations. Daily percutaneous nerve conduction tests were carried out, and electromyography was performed at regular intervals by the department of neurology.

Peripheral facial palsy in pregnancy is not included in the idiopathic material, since this form of facial palsy occurs preferentially at definite stage in pregnancy, may therefore have a specific pathogenesis (Mair et al, 1972) and also, by definition, occurs only in a restricted age group. The Melkersson-Rosenthal syndrome, which constitutes a specific clinical complex, is likewise excluded from the Bell's palsy group.

All patients diagnosed as having idiopathic peripheral facial palsy, with the exception of a small group (8) of diabetics, received a 10-day course of adrenocorticotrophic hormone.

Medical colleagues were contacted at regular intervals throughout the five year period and reminded of our interest in facial palsy.

Referral rates in the latter 3 years of the investigation, 1969-1971, were considerably higher than in the first 2, largely on account of an initial reluctance on the part of physicians to send patients the often long distances

to our hospital for what is widely considered a "benign" illness. The referral rates for the years 1969 through 1971 are therefore considered to give a more accurate index of the incidence of facial palsy in our part of the world.

RESULTS

In the five-year period, 1967-1971, 173 individual peripheral facial palsies were investigated. The annual totals for the various aetiological groups are shown in Table I. Of the fourteen cases of fracture of the temporal bone, three were of immediate onset and were decompressed, the nerve being found constricted by bone fragments in the pyramidal region. The two cases of operative trauma: a revision mastoidectomy and revision parotidectomy, had both received their facial palsy 10 years earlier at prior surgery, and presented only a temporary deterioration of facial nerve function following reoperation. All cases of herpes zoster paralysis were suspected clinically, either on the basis of the dermatological findings, or the presence of auditory and vestibular symptoms, the diagnosis being confirmed by the demonstration of rising antibody titres. In no instance was serological evidence of zoster infection found which was not suspected clinically.

Of the 134 patients with Bell's palsy, 77 were male and 57 female, 2 females having simultaneous bilateral palsies. Fourteen pa-

Table II *Idiopathic facial palsy, number of cases and incidence rates per 100 000 of the population per year, for both the entire five-year period and for the latter three years of the study, presented as a function of age*

The incidences for the period 1969-71 are presented separately for both sexes in addition to the total incidences

Age group	1967-1971		1969-1971			
	Number	Incidence	Number	Incidence		
				Males	Females	Total
0-9	7	3.9	5	1.8	7.6	4.7
10-19	13	8.3	9	6.2	13.1	9.5
20-29	28	19.7	22	26.2	25.4	25.8
30-39	22	23.0	15	32.5	18.0	26.1
40-49	18	18.3	10	19.1	14.5	17.0
50-59	23	24.7	18	31.3	33.2	32.2
60-69	12	17.8	10	29.5	19.9	24.7
70+	13	24.8	11	41.7	29.3	35.0
0-70+	136	15.4	100	19.3	18.4	18.8

tients (10.5%) had a family history of facial palsy, and 8 (6%), (5 females and 3 males) gave a history of previous episodes either on the same or the contralateral side. Thirteen patients (9.7%) had a lingua plicata with out otherwise fulfilling the diagnostic criteria for the Melkersson-Rosenthal syndrome, although one of these had previously had a paralysis on the same side.

The distribution of the idiopathic facial palsies according to the patients' age is shown in Table II. Incidences have been calculated on the basis of population statistics and are expressed as the number of palsies per 100 000 per year. These are shown for the entire five year period and separately for the latter 3 years of the study. As mentioned previously, the figures for the period 1969-71 are regarded as giving a more accurate estimate of the true incidence of Bell's palsy in this area. The total number of palsies and the incidences for the different age groups are presented graphically in Fig. 1 for this three year period. Incidences are low in the first two decades of life, increase markedly after 20 years and show no indication of a falling off in the older age groups. In Table II the incidences for Bell's palsy in the period 1969-71 are also shown separately for both sexes.

There would appear to be a tendency for a higher incidence amongst females in the younger age groups, but the differences are not statistically significant.

The seasonal distribution of the 134 Bell's palsies is shown in Table III. An ordinary chi square test gives $P=0.09$, but the Edwards test of cyclic trend demonstrates no correlation between the onset of the palsies

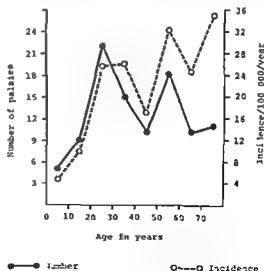


Fig. 1 The number of Bell's palsies and the incidences per 100 000 of the population per year plotted against age groups in decades for the period 1969 to 1971.

Table III Seasonal distribution of Bell's palsy ($n=134$, two cases of uncertain onset)

	1967	1968	1969	1970	1971	Total
January		1	1	3		5
February	1	2	5	8	3	19
March			3	5	1	9
April		2	5	3	1	11
May	1	3	3	5	2	14
June		1	2	3	2	7
July	2		3	2	3	10
August	1	1	2	2	3	9
September	2	1	3	3	3	13
October	3	4	3	1	3	14
November	2	4	1	3	5	15
December		3	2	1	2	8
Total	12	23	33	38	28	134

and the month of the year. On combining the data for age and seasonal distribution (Table IV) no evidence is found that seasonal incidence varies with age group.

DISCUSSION

The term Bell's palsy probably includes several different pathogenetic mechanisms all of which may produce the same clinical picture of a lower motor neurone facial paralysis (Alter 1963, Sade 1972). The facial palsies of the Melkersson-Rosenthal syndrome, pregnancy and herpes zoster infection are neurologically indistinguishable from the group of idiopathic paralyses.

In the present material the Melkersson-Rosenthal syndrome accounts for 3.5% of the

total number of peripheral facial palsies. This figure is surprisingly high when compared with other series in the literature. Both in California (Adour & Swanson 1971) and in southern Sweden (Lagerholm & Toremalm 1971) no cases were encountered in comparable series and in Holland only one case was diagnosed in a study of 750 peripheral facial palsies (Devriese & Pelz, 1969). The occurrence of a peripheral facial palsy in a patient with a history of recurrent facial swelling and typical histology is regarded as diagnostic even although lingua plicata is lacking since the latter is found in only approximately one third of cases of this syndrome (Hornstein 1970). It has also been proposed that lingua plicata in the presence of recurrent facial paralysis should be regarded as belonging to the Melkersson-Rosenthal syndrome even though the most important diagnostic feature, recurrent facial swelling, is absent (Ekblom 1950). These latter criteria were however not applied to the present series.

The idiopathic form accounts for between 75% and 85% of all cases of peripheral facial palsy in the literature (Peitersen & Andersen 1967, Lagerholm & Toremalm 1971, Adour & Swanson 1971, Hauser et al., 1971) only Cawthorne (1951) reporting a significantly lower incidence due to the large number of traumatic cases in his series. In only one report has Bell's palsy accounted for a significantly higher percentage of cases with 93.6% of patients being classified under this diagnosis (El Ebiary, 1971) but otological examination does not appear to have been performed routinely in this study. The present figure of 78.6% is lower than many other reports in the literature but most authors do not differentiate the pregnancy and Melkersson-Rosenthal groups from the general diagnosis of Bell's palsy.

The recurrent form is variously reported as from 2.6% (Adour & Swanson 1971) up to 15.2% (Boddie, 1972) of all cases of idiopathic facial palsy. The latter author concluded without statistical evidence that the

Table IV Distribution of Bell's palsy by age group and season of the year

	Age group			Total
	0-29	30-59	60	
May-October	25	29	13	67
November-April	22	34	11	67
Total	47	63	24	134
June-November	24	31	13	68
December-May	23	32	11	66
Total	47	63	24	134

Table V *Incidence of recurrent Bell's palsy*

Author	Recurrent	Total	% Recurrent
Park & Watkins 1949	31	440	7.0
Cawthorne & Haynes, 1956	33	347	9.5
Dalton, 1960	5	97	5.2
Devriese & Pelz, 1969	46	440	10.5
Adour & Swanson 1971	8	308	2.6
Lagerholm & Torealm, 1971	12	144	8.3
El Ebiary, 1971	49	528	9.0
Boddie, 1972	25	165	15.2
Present series	8	134	6.0
Total	217	2 603	8.3

true incidence of recurrent Bell's palsy was probably less than 5%. A review of several series in the literature (Table V) gives a mean incidence of 8.3% for recurrent idiopathic facial palsy. Devriese & Pelz (1969) reported a statistically significant greater recurrence rate in males, whereas El Ebiary (1971) came to the diametrically opposite conclusion. Unfortunately, the sex distribution of recurrent Bell's palsy is not mentioned in the majority of reports in the literature.

Idiopathic facial palsy is generally considered a disease of the younger, middle age group, the decades from 20 to 50 showing the highest incidence (Park & Watkins, 1949, Cawthorne & Haynes, 1956, Taverner, 1959, Gregg 1961, Lagerholm & Torealm, 1971). A strikingly different distribution has been

reported by El Ebiary (1971) from Alexandria, where 44.8% of cases occurred in the age group younger than 20 years. However, in none of these studies was the incidence correlated with the age distribution of the population under consideration. The present material demonstrates that the incidence in northern Norway is lowest in the first two decades, increases markedly after 20 years and remains at a high level thereafter. Only Hauser et al (1971) have treated their data in a similar manner and found a strikingly similar age distribution. In agreement with the report of Alter (1963) the present study shows a slightly greater incidence amongst females in the younger age groups, but this difference is not statistically significant.

Epidemiological studies which are based on the referral of cases to a central, specialty department are notoriously unreliable, as evidenced by the differences between our three- and five-year statistics. The incidence of 18.8/100 000/year for the three-year period 1969-1971 in northern Norway is of a similar order of magnitude as several other reports in the literature (Table VI). A common rate of 15/100 000/year is obtained when the data for the six regions included in Table VI are combined. The report from Sweden (Lagerholm & Torealm, 1971) gives a significantly lower incidence (X^2 10.09), whilst the series from Minnesota (Hauser et al, 1971) is significantly higher (X^2 26.49) than the calcu-

Table VI *Incidence of Bell's palsy in various regions*

Region	Author	Incidence	Study	Observed	Expected	χ^2
Belfast	Gregg	16.0	4 400	661	660.0	0.00
England	Melotte	16.6	165	20	24.8	0.71
California	Adour & Swanson	13.5	1 800	243	270.0	2.70
Sweden	Lagerholm & Torealm	11.5	1 250	144	187.5	10.09
Minnesota	Hauser et al	22.8	507	121	76.1	26.49
North Norway	Mair & de Graaf	18.8	531	100	79.7	5.17
Total			8 653	1 298	1 298.1	45.16

Common rate $1 298/8 653 \times 100 = 15.0/100 000$ year

Study is the product of the number of years and the population in thousands in each series. Observed and Expected refer to the number of palsies in each study.

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October	3	4	3	1	3	14
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MALIGNANCY OF ACINIC CELL TUMOURS ELUCIDATED BY MICROSPECTROPHOTOMETRIC DNA ANALYSIS

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Abstract: "Acinic cell tumour" is nowadays generally considered as a biological malignant tumour type but the often existing pronounced benign histological features have raised the question unless beside the malignant tumours of this type there also exists a benign variety. In order to study this problem microspectrophotometric determinations of the nuclear DNA content of Feulgen stained cells of an acinic cell tumour without malignant histological features have been performed and compared with control cells and tumour cells from a biological benign and a biological malignant salivary gland tumour. The microspectrophotometric DNA analysis performed in the present study showed that the nuclei of an acinic cell tumour with histologically benign features exhibited significantly higher DNA values than normal control cells

and the cells from the benign tumour (a monomorphous adenoma). On the other hand the microspectrophotometric analysis showed that the nuclear DNA content of the "acinic cell tumour" cells was increased to the same level as in the cells of a poorly differentiated adenocarcinoma. Thus the results of the DNA analysis argue against the existence of a benign variety of the acinic cell tumour which therefore irrespective of the histological features should be considered as a malignant type of tumour.

Until 1953 when Buxton et al showed that "acinic cell tumour" could have a clinically malignant course its histologically appearance led to its being denoted as a benign type. This is evident from the various synonyms in the literature used for it such as "epithelioma glandulaire" (Masson 1924) "acinare Adenome" (Lang, 1929) "parathyreoidea ähnliche Geschwulst" (Franssen 1932-33) "Huckel 1930) "serous acinar adenoma" (Skorpiol 1940)

"glycogen rich clear-cell adenoma" (Corridan 1956)

The cytological picture is often dominated by cells denoted as acinic cells because of their great resemblance to normal acinar cells. In view of the presumed benign course and the dominant type of cell the name "acinic cell adenoma" was introduced (Godwin & Colvin 1948). Studies during the past 15 years however, have disclosed that the "acinic cell tumour" can metastasize and cause death in the tumour disease. The terms "acinic cell adenocarcinoma" (Godwin et al, 1954) and "acinic cell carcinoma" (Beahrs et al, 1960) (Grage et al, 1961) therefore were proposed.

In "acinic cell tumours" the often pronounced benign morphological features have raised the question, whether there is a benign variety of this tumour. Thus, Kleinsasser et al (1967) have stated that there exist undoubtedly benign and malignant forms of acinic cell tumour, but that it is not possible on the basis of histological features to separate the benign and malignant types.

Seifert (1966) combined "acinic cell carcinoma" with "acinar adenoma" and "alveolar adenoma" in a group which he called "Acinuszelltumoren" and denoted the group as "faktitiv maligne". Kleinsasser et al and other authors who believe in a benign and a malignant form of this tumour type use the designation

"acinic cell tumour" whereas authors who believe that there is no definitely benign group of this tumour denote it as "acinic cell carcinoma"

Increased nuclear DNA content has been shown to be a characteristic feature of malignant salivary gland tumour cells (Eneroth & Zetterberg, in press). In order to add information to the question of malignancy in acinic cell tumours with histologically benign structures an analysis of the nuclear DNA content in the individual cell nuclei of an acinic cell tumour without malignant histological features has been performed.

METHODS

A parotid tumour with histological structures typical for an acinic cell tumour without malignant histological structures was selected for the analysis. For the microscopic examination of the tumour routine histological techniques were employed.

For the microspectrophotometric analysis of the nuclear DNA content imprint preparations were made from the cut fresh surface through the tumour tissue immediately after the removal of the tumour. Haemocytometer glass slides were used. The imprint preparations were immediately fixed in a freshly prepared mixture of ethanol and acetone (1:1) for 30 minutes and thereafter stored in the refrigerator (+4°C) until the staining was performed according to the Feulgen procedure (Eneroth & Zetterberg, in press). Human lymphocytes from peripheral blood and cells from normal parotid gland were used as control cells. Furthermore cell populations from a biological benign salivary gland tumour (a cell rich monomorphic adenoma) and from a clearly malignant salivary gland tumour (a poorly differentiated adenocarcinoma) were microspectrophotometrically analysed for comparison. The DNA content of the Feulgen-stained individual cell nuclei was determined by absorption measurements in a rapid scan-

ning microspectrophotometer (Lomakka, 1965) at the wavelength of 546 nm.

RESULTS

The histological slides were studied by the following methods of staining: hematoxylin-eosin, van Gieson and PAS.

The tumour, that was macroscopically circumscribed and measured 2 cm in diameter, was built up by round and polyhedral cells arranged in equally sized lobules separated by thin septa of connective tissue (Fig. 1). No evident glandular lumina were observed.

The histological pattern of the tumour was consistent with an acinic cell tumour. The only differential diagnosis considered was that of an oncocytoma. The cytoplasm of the tumour cells were, however, mostly granular and faintly basophilic, and in small areas there also existed clear cells. PAS-positive material was found in many cells, strongly indicating the "acinic cell tumour" diagnosis.

The tumour was mostly monomorphic without cellular atypia but in some parts one could find a slight degree of nuclear abnormalities in the form of single, somewhat enlarged hyperchromatic nuclei. Histomorphological criteria of malignancy were, however, lacking. No obvious invasion could be demonstrated.

Microspectrophotometric measurements of randomly selected Feulgen stained nuclei of the imprint preparations are illustrated in Fig. 2. As control cells for the Feulgen staining procedure human lymphocytes from peripheral blood were used. The mean Feulgen value of the human lymphocytes was given the value 2c, which denotes the normal diploid DNA content. Each measured value was related to its corresponding staining control and expressed in the 2c units. It is clear from the figure that the nuclei from the "acinic cell tumour" contain more DNA than the lymphocytes, the normal parotid gland cells and the benign monomorphic adenoma cells, which all



Fig 1 Acinic cell carcinoma of the parotid gland
Photomicrograph $\times 400$

exhibit the normal diploid DNA content, 2c. On the other hand the similarity between the "acinic cell tumour" and the clearly malignant poorly differentiated adenocarcinoma from the

parotid gland is evident, the majority of the nuclei from these two tumour types contain between 2.2c and 2.5c units of DNA i.e. a clearly hyperdiploid value.

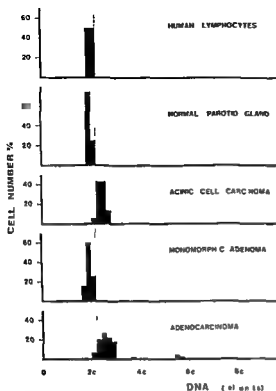


Fig 2 Frequency histograms of nuclear DNA quantity (Feulgen positive material). Five different cell types were analysed: human lymphocytes from peripheral blood (staining controls), cells from a normal parotid gland, from an acinic cell tumour, from a monomorphic cell rich adenoma of the palate and from a poorly differentiated adenocarcinoma of the parotid gland. Each histogram is based on the microspectrophotometric analysis of between 50 and 75 randomly selected cells. The DNA values are expressed in relation to the staining control (human lymphocytes) which was given the value of 2c.

DISCUSSION

The microspectrophotometric DNA analysis performed in the present study showed that the cell population of an acinic cell tumour with histologically benign features exhibited significantly higher DNA values than normal control cells and the cells from a benign tumour (a monomorphic adenoma). Despite the histologically benign features of the acinic cell tumour in the present study, the microspectrophotometric analysis showed that the nuclear DNA content of the tumour cells was increased to the same level as in the cells of a

clearly malignant salivary gland tumour (a poorly differentiated adenocarcinoma). These two tumours both had a modal DNA value between 2c and 2.5c, suggesting the existence of an aneuploid stemline in the hyperdiploid region.

From a clinical follow up study of a large tumour material for a long period (Blanc et al, 1971) it is obvious that the highly differentiated mucoepidermoid tumour and the acinic cell tumour are more low-grade malignant than the other malignant salivary gland tumours. The existence of a benign variety of these two tumour types has also been questioned. Concerning mucoepidermoid tumours with highly differentiated structures Eneroth & Zetterberg (1973) have shown that this tumour type exhibits higher DNA values than normal control cells and should despite the benign histological features be considered as malignant and therefore be denoted as a carcinoma. Concerning the acinic cell tumour without histologically malignant features the present study indicates that also acinic cell tumour despite the benign histological features exhibits DNA values characteristic of cells of a clearly malignant salivary gland tumour. These facts argue against the existence of a benign variety of the acinic cell tumour. Thus, the acinic cell tumour irrespective of the histological features, should be considered as malignant and therefore be denoted as 'acinic cell carcinoma'.

ZUSAMMENFASSUNG

Ein Acinuszelltumor wird nunmehr allgemein als ein biologisch maligner Tumortyp angesehen aber die oft vorliegenden ausgesprochen benignen histologischen Strukturen führen zu der Frage ob neben den malignen Tumoren dieses Typs nicht auch eine benigne Variante besteht. Zum näheren Studium dieses Problems wurden mikrospektrophotometrische Bestimmungen des nukleären Gehalts an DNS bei Feulgenscher Nuklealfärbung eines Acinuszelltumors ohne makroskopisch histologische Strukturen durchgeführt und mit Kontrollzellen und Tumorzellen eines biologisch benignen und eines biologisch malignen Speicheldrüsentumors verglichen. Die mikrospektrophoto-

metrische DNS-Analyse zeigte dass die Nuklei eines Acinuszelltumors mit histologisch benignen Strukturen signifikant höhere DNS-Werte aufwiesen als normale Kontrollzellen und die Zellen des benignen Tumors (eines monomorphen Aderoms). Andererseits zeigte die mikrospektrophotometrische Analyse dass der nukleare DNS-Gehalt der Zellen des Acinuszelltumors im gleichen Ausmass erhöht war wie in der Zellen eines gering differenzierten Adenokarzinoms. Die Resultate der DNS-Analyse widersprechen somit der Existenz einer benignen Variante des Acinuszelltumors. Ein solcher Tumor muss daher ohne Rücksicht auf die histologischen Strukturen als ein maligner Tumortyp angesehen werden.

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THE EFFECTS OF SYMPATHETIC NERVE STIMULATION ON THE TRACER DISAPPEARANCE RATE AND LOCAL BLOOD CONTENT IN THE NASAL MUCOSA OF THE CAT

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Abstract The effects of sympathetic nerve activation on tracer disappearance rate and local blood content in the cat nasal mucosa were studied by measuring local disappearance of ^{125}I - and changes in gross pulse rate from ^{125}I labelled serum albumin monitored over the nose. Major changes in the tracer disappearance rate were seen in the frequency range of 0.5-6.0 imp/sec while similar changes in the local blood content occurred at 0.1-0.5 imp/sec indicating a functional differentiation in the vasoconstrictor control of the exchange and capacitance vessels during low sympathetic tone. The observed responses were shown to be due to activation of sympathetic adrenergic nerves and receptors of the α type. No evidence of vasodilator influence due to stimulation of β receptors or sympathetic cholinergic vasodilator fibers was found.

The effects of sympathetic nerve activation on the local blood content of the nasal vascular bed has previously been studied with rhinomanometric techniques, where changes in the lumen of the nasal passages yielded indirect measurements of changes in the blood content (capacitance function) of the mucosa (Tschalussow, 1913; Malcomson, 1959; Franke, 1966; Hall & Jackson, 1968; Richerson & Seeborn, 1968; Rooker & Jackson, 1969). These experiments suggest a strong sympathetic influence on the capacitance vessels although a direct method for the study of the capacitance function has so far not been demonstrated.

Little is known about the type of sympathet-

ic receptors in the nasal mucosa and no information is available on the sympathetic influence on the exchange vessels in the nasal vascular bed. Therefore, knowledge of the interaction between events in the exchange and capacitance vessels is still not at hand. The present investigation was undertaken to study these events as reflected in the effects of graded sympathetic nerve activation on tracer disappearance rate and local blood content in the nasal mucosa. We also wanted to classify the receptors mediating the vascular responses to sympathetic nerve activation. It might be observed that in procedures providing quantitative information about changes in the vascular bed, the various vascular sections should preferably be studied simultaneously (cf. Mellander & Johansson, 1968).

Surgical Procedures

The experiments were performed on 49 cats (2.4-4.3 kg) anesthetized with chloralose urethane (50 mg/kg + 100 mg/kg i.v.) or, in three cases where denervation effects were studied, chloralose (60 mg/kg i.v.) after induction with ether. The trachea was cannulated. The pressure in the femoral artery was measured by a Statham pressure transducer (P23A) and recorded on a Rikadenki multichannel recorder. Rectal temperature was measured and was kept constant at 38°C by heating lamps. The cervical sympathetic trunk was dissected free

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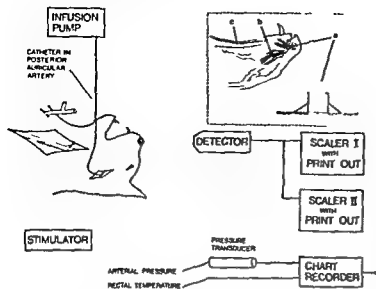


Fig 1 Drawing showing experimental procedure. The lead shields masking the nasal cavities are not shown. Inset represents sagittal section of the head of the cat and the application technique with the polyethylene tube fixed over the de-epithelized area on the inferior concha (a) middle concha (b) nasopharynx (c).

from the vagal nerve and transected. Sympathetic stimulation was performed in the cranial direction using a bipolar silver electrode. The stimuli were monophasic square wave pulses delivered by a Grass model S4 stimulator. Stimulation frequencies were varied between 11–12 imp/sec in random order. In the experiments where α -adrenergic blocking agents, isoproterenol and papaverine were used, the posterior auricular artery was cannulated for close intra-arterial infusion into the external carotid artery. The head of the cat was immobilized by means of a steel rod inserted between the jaws and secured in place by dental acrylic.

Disappearance Measurements

The tracer disappearance measurements were performed with an easily diffusible water-soluble radioactive tracer, ^{125}I , as iodide (Kety, 1949). The tracer was obtained as an isotonic, carrier free solution in concentrations of 300 $\mu\text{Ci } \mu\text{l}$ (AB Atomenergi, Nyköping, Sweden). In preliminary experiments volumes of 0.5–1.5 μl of the isotope were injected subepithelially in the mucosa of the anterior two-thirds of the inferior concha on one side. The mucosa was visualized by widening of the vestibulum of the nose with a speculum and inspection through a Leitz operation microscope. A glass

capillary with a tip diameter of 50–100 μm was used as an injection cannula. The capillary was attached with a polyethylene tube (PE 20) to a Hamilton 10 μl syringe, fixed to a stand. The injection time was 3–5 min. In 2 cats the radioactive tracer was injected together with an intravital dye (5% Fluorescein sodium, or 6% Procion navy blue) and the injection area was examined histologically to evaluate the spread of the tracer. In a second series of experiments using 15 cats the injection technique was changed to an application technique. The epithelium at the application site was removed and a small polyethylene tube (PE 90) applied and fixed over the exposed area with an adhesive (Nobecutan, Bofors), thus creating an open well with the subepithelial tissues in the bottom (Fig 1). About 1 hour later 0.5–1 μl of the tracer was placed in the cavity. The disappearance of the applied depot was monitored by an external scintillation detector secured in a fixed relation to the nose. The distance between the depot and the detector was 5–15 cm. Apart from the nose, the animal was shielded by 3 mm lead plates. The detector output was fed into two recording channels, each containing a single channel pulse height analyzer and two scalers with digital print out. A schematic drawing of the setup is shown in Fig 1. For further technical details concerning

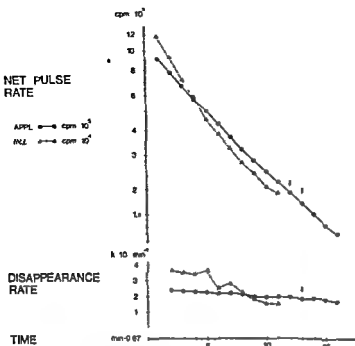


Fig 2 Disappearance rates of a ^{125}I -depot injected subepithelially and a locally applied depot in the same area. Resting conditions. The disappearance rate is illustrated in two ways, both as a semilogarithmic plot of the net pulse rate of the depot against time and as k values taken from the slopes between two consecutive readings. The k value at the arrow was derived from the slope between the two points in the net pulse rate curve indicated with arrows.

the apparatus, see Bolme & Edwall (1970). Radioactivity was counted for periods of 40–60 sec, and measurements started immediately after the application of the tracer. After each run the total final background was determined (cf. Odeblad et al., 1959). The net pulse rate (gross pulse rate minus background) was plotted semilogarithmically against time. The disappearance rate (k -value) which represents the fractional elimination of the depot per minute was calculated as follows (Kety, 1949):

$$k = (\log C_1 - \log C_2) / 0.4343 (t_2 - t_1)$$

where C_1 and C_2 are the recorded net pulse rates per minute of the depot at the time t_1 and t_2 . The calculations were made on a computer for each time interval. For further details concerning the calculations see Edwall (1971).

Reduction in tracer disappearance rate ($k\%$) was calculated from the expression $k\% = 100 (k_{\text{contr}} - k_{\text{stim}}) / k_{\text{contr}}$ where k_{contr} and k_{stim} are the means of four k -values during the preceding control period and the stimulation period, respectively.

Measurements of Local Blood Content

In 7 cats, changes in total blood volume of the nose were studied separately or simultaneously with the disappearance measurements. A lead shield of 5 cm thickness fitting tightly around the head and the nose of the cat had a narrow slit over the right side of the nose and shielded the rest of the cat from the detector. ^{131}I -albumin (1–2.5 mCi in 5 ml) was injected, intravenously. Gross pulse rate was measured as described above. When double tracers were used, the pulse rates in both recording channels were determined separately. Appropriate corrections were made for the overhearing of ^{131}I in the ^{125}I channel. Reductions in gross pulse rate during stimulation were expressed as a percentage of the maximal reduction obtained at 6 imp/sec.

RESULTS

Tracer disappearance measurements during resting conditions

Following injection of the tracer solution into the mucosa the disappearance rate (k value) varied considerably in different injections.

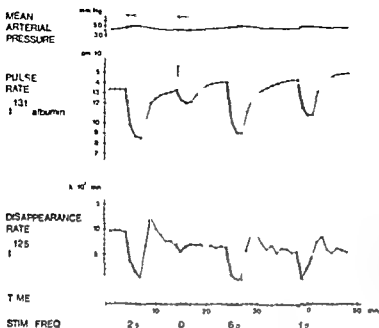


Fig 3 Influence of sympathetic nerve stimulation on the disappearance rate from a locally applied depot of ^{125}I and the gross pulse rate of ^{131}I albumin measured over the right nasal cavity

The deep injections or injections where a slight bleeding occurred regularly resulted in a polyexponential disappearance curve with an initially high disappearance rate during the first 5–10 min which thereafter decreased almost monoexponentially down to a final background. Results from these curves could only be evaluated qualitatively as changes occurred in the disappearance rate even under resting conditions.

Subepithelial injections resulted in an almost monoexponential disappearance curve with a high disappearance rate for about 10 min down to the background (Fig 2). This curve was most often found after the first injection or when the thin epithelial layer was seen to bulge slightly during the injection. Histological examination of the mucosa after a corresponding injection of an intravital dye revealed a subepithelial spread of the depot and penetration down into the submucosa. No penetration through the epithelial layer could be seen.

A comparison between a subepithelial injected depot and a depot applied immediately afterwards at the same site is shown in Fig 2. The λ value was initially higher in the injected depot than that from the local application but gradually decreased.

In the experiments where the application technique was used (15 cats, 72 applications) the disappearance curves showed a rapid monoexponential disappearance rate for 15–30 min and came down to a low final background recording which was not changed noticeably when the cavity was rinsed with saline. When the application was made immediately after the preparation a slight decrease in the λ values was noted as seen in Fig 2 where the λ value decreased from 0.23 to 0.17 in 11 min.

Local blood content during resting conditions

Provided that no bleeding occurred within the area seen by the scintillation detector no noticeable change occurred in the gross pulse rate during a 30–50 min period.

Effect of sympathetic nerve stimulation

In all experiments sympathetic nerve stimulation in the frequency range of 0.1–6 imp/sec induced frequency dependent reductions in the λ value and local blood content.

Fig 3 shows an experiment where both tracers were studied simultaneously. Stimulation at 2.5 imp/sec reduced the disappearance rate by 83% while the subsequent low fre-

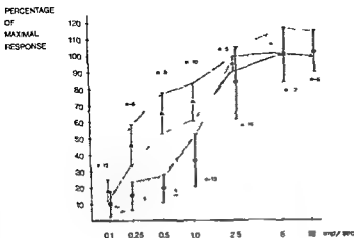


Fig 4 Influence of sympathetic nerve stimulation on tracer disappearance rate (shaded curve) and the local blood content (unshaded curve) in the nasal mucosa. Changes expressed as percent of the reduction at 6 imp/sec. Mean and deviation and number of observations are shown.

quency stimulation at 0.1 imp/sec induced a reduction of 14%. Stimulation at 6 imp/sec reduced the disappearance rate by 90%. During the subsequent recovery after stimulation frequencies above 1 imp/sec there was a transient overshoot followed by return to control values. At frequencies of 1 imp/sec or below an escape was seen.

The gross pulse rate of ^{125}I albumin was simultaneously reduced and a maximal reduction was seen at 6 imp/sec and a clearcut reduction at 0.1 imp/sec which was 25% of the maximal reduction. No escape was found in any of the experiments. When oximetazoline (Nezeril, Draco) was applied to the mucosa and compared with sympathetic stimulation it was found that the reduction in pulse rate was 80–90% of that following supramaximal stimulation.

The relationship between stimulation frequency and the percentage change in k -values from disappearance measurements with the application technique is demonstrated in Fig 4 (shaded curve). It is seen that a frequency-dependent response was observed between 0.5 and 6 imp/sec.

The responses to repeated stimulation with the same frequency varied less within the same animal than between animals. The same qualitative results were also obtained in disappearance measurements from the locally injected depots.

The effect of sympathetic nerve stimulation on the local blood content is also illustrated in Fig 4. A pronounced increase in the magnitude of the responses was observed between 0.1 and 0.5 imp/sec. The decrease in the pulse rate was 22, 55 and 75% at 0.1, 0.25 and 0.5 imp/sec respectively. An almost maximal response was obtained at 2.5 imp/sec. Comparing the frequency response curves for tracer disappearance rate and local blood content (Fig 4), it is seen that the frequency response curve for the local blood content is shifted to the left in relation to the corresponding curve for the disappearance rate.

Sympathetic stimulation after an α -adrenergic blocking agent

After infusion of dihydroergotamin (Orstanorm, Sandoz, 0.2 mg/kg) in 2 cats the effects of stimulation at frequencies below 1 imp/sec were almost completely abolished. At higher stimulations a partial block was found. Phenolamine 5 mg/kg (Regitine, Ciba) was infused in four experiments and resulted in a complete block at all stimulation frequencies.

In order to investigate the presence of β receptors the α receptors were blocked. Stimulation after a complete block did not induce any significant increase in the disappearance rate (Fig 5, 3). Infusion of a β adrenergic agent, isoproterenol, in doses of 0.01 μg –0.30 $\mu\text{g}/\text{min}$ also failed to influence the disappear-

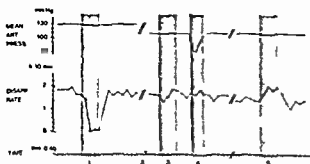


Fig 5 Influence of an α -adrenergic blocking agent on disappearance rate of ^{125}I during sympathetic stimulation and the effects of isoproterenol and papaverine Cat 3.2 kg

- 1 Stimulation 6 V, 1 msec, 12 imp/sec
- 2 Phentolamine i.a. infusion 5 mg/kg
- 3 Stimulation 6 V, 1 msec, 12 imp/sec
- 4 Isoproterenol i.a. infusion 0.30 $\mu\text{g}/\text{min}$
- 5 Papaverine i.a. infusion 0.30 mg/min

ance rate. The latter dose caused a fall in the systemic blood pressure which can be seen in Fig 5 4.

The response in the local blood content upon sympathetic stimulation could also be blocked by phentolamine as seen in Fig 6. A partial blockade was seen after infusion of 2.5 mg phentolamine while a complete block was obtained even at high frequencies after 5 mg/kg. No increase in the local blood content was then noted upon repeated stimulation.

Acute effects of sympathetic denervation

To obtain information about the influence of sympathetic nervous tone during resting condi-



Fig 6 Influence of an α -adrenergic blocking agent on the gross pulse rate of ^{125}I albumin during sympathetic stimulation Cat 2.5 kg 1 Phentolamine 2.5 mg i.a. infusion 2 Phentolamine 10 mg i.a. infusion

tions the sympathetic nerve was transected during disappearance measurements on 4 cats and during measurements of local blood content in 1 cat. Such denervation consistently resulted in a slight increase (Maximally 16%) in the rate of tracer disappearance (λ -value) and an increase in the local blood content.

DISCUSSION

No attempt has been made to transform the disappearance rate (λ -value) of iodide into values of total blood flow since other factors, such as changes in capillary exchange surface area and capillary flow distribution and velocity, are of quantitative importance (cf Melander & Johansson, 1968). Furthermore, the abundant arterio-venous anastomoses within the nasal vascular bed as described by Dawes & Prichard (1953) would invalidate calculations of total blood flow based on λ -values since these are dependent on blood flow through the exchange vessels (Kety, 1960). For example, a redistribution of blood flow from shunt vessels to exchange vessels without changing total blood flow would increase the λ values.

Among other factors that may influence the disappearance rate is the trauma induced by the injection needle, and the injected volume (Sejrsen, 1971). Though the present technique was aimed at reducing these factors, the rapid initial disappearance rate seen after the deep injections is probably a result of the injection trauma and possibly also a consequence of heterogeneous tissue perfusion within the depot (cf Edwall & Kindlová, 1971). The slow disappearance rate seen during the monoexponential late part of these curves might be due to diffusion barriers within the depot. The application technique was developed to overcome the difficulties with the injection technique. The slight decrease in the λ values seen at local application immediately after the preparation is probably also the effect of trauma since this effect was not noticed when the application was made 1 hour later. A possible

reason for the monoexponential disappearance curve regularly found with the application technique could be the existence of rate limiting barriers for diffusion between the depot and the exchange vessels. However, as increases in disappearance rate were found when the blood vessels were dilated with papaverine, this limitation would then only exist at higher flow rates.

Using ^{131}I labelled albumin we also obtained data on the local blood content of the nasal mucosa. Rhinomanometry has previously been used to record changes in the lumen of the nasal passages and thus indirectly measure changes in the blood content of the mucosa. An assumption important for this technique is that the quantity of interstitial or intracellular fluid is not altered at the same time, or measurements are not disturbed by excessive nasal secretion (cf Drettner, 1963). With the method described here these difficulties are avoided and the measured radioactivity expresses relative changes in plasma volume in the tissues seen by the detector. If it is assumed that the hematocrit is not changed during the experimental procedures, then the measured changes represent changes in the regional blood volume. Jodal & Lundgren (1970) studying intestinal blood volume distribution, did not find any significant change in the relative distribution of red cells and plasma volumes during nervous vasoconstriction and it seems reasonable to assume a similar relationship in the nasal vascular bed.

To what extent measured changes represent changes solely in the nasal vascular bed is difficult to state with certainty and depends on whether the detector monitors events from surrounding tissues. However, since the local application of a vasoconstrictor drug on the nasal mucosa induced a reduction which was 80–90% of that obtained following maximal sympathetic nerve stimulation, it appears likely that most of the changes would reflect alterations in the blood content (capacitance function) of the nasal mucosa.

The maximal effects of sympathetic nerve

stimulation on tracer disappearance rate, corresponding to around a 70% reduction of the k -values, were obtained with frequencies of about 6 imp/sec. This magnitude of change is similar to that seen in oral tissues and skeletal muscle as reported by Edwall & Kindlova (1971), who used similar tracer disappearance techniques.

The major changes in the exchange function were seen in the frequency range of 0.5–6.0 imp/sec while similar changes in the capacitance function occurred already at 0.1–0.5 imp/sec. This effectiveness of low frequency stimulation on the capacitance function is in agreement with results obtained with a rhinomanometric technique in the dog (Franke, 1966).

Our results showing a dissociation in sympathetic response between the exchange and capacitance function might be explained by the very rich adrenergic innervation around the venous sinusoids and the less pronounced innervation within the subepithelial and periglandular vascular plexus as found in histochemical studies of the adrenergic innervation of the nasal mucosa (Dahlstrom & Fuxe, 1967; Ånggård & Densert, to be published).

A relative difference in vasoconstrictor response between the resistance and capacitance vessels of the cat's leg has further been described by Mellander (1960) and was partly explained by the difference in the wall/lumen ratio of the resistance (mainly thick-walled arterial vessels) and the capacitance vessels (mainly thin walled veins). This explanation might also apply to the nasal mucosa where wide thin walled veins occur abundantly.

Furthermore, the shift to the right of the frequency-response curve for the tracer disappearance rate could also be explained by a redistribution of blood flow from shunts to exchange vessels, since shunts in skin have been shown to be highly sensitive to low sympathetic vasoconstrictor activity (cf Mellander & Johansson 1968).

Hall & Jackson (1968), in a rhinomanometric study of the effects of α - and β adrenergic

agonists, demonstrated the presence of α receptors in the nasal mucosa while the existence of β receptors was considered to be doubtful. In the present study the effects of α -receptor blocking agents clearly demonstrate that the observed responses in the exchange and capacitance vessels are due to activation of sympathetic adrenergic nerves and receptors of the α type. When a complete blockade of the α -receptors was obtained, no increase in the exchange or capacitance function was observed during sympathetic nerve stimulation. Hence, there is no evidence for a vasodilator influence due to stimulation of β receptors or sympathetic cholinergic vasodilator fibers in the nasal vascular bed. Ishii & Toriyama (1972) observed in a histological study of the human nasal mucosa a cholinergic innervation independent of the sphenopalatine ganglion. This observation if applicable to the cat nasal mucosa, would then not represent sympathetic cholinergic vasodilator fibres affecting the exchange- or capacitance vessels. Cutting the cervical sympathetic nerve did not increase the disappearance rate more than 16% indicating a resting impulse frequency of less than 0.5 imp/sec during the present conditions. This low sympathetic tone might be attributed to the narcosis as suggested by Rooker & Jackson (1969) because in the present study, a hyperaemia of the external nose was noted during the induction of the narcosis.

Experiments on animals and humans have shown that the tonic discharge rate of autonomic sympathetic fibers under resting conditions is about 1-3 imp/sec and rarely exceeds 10 imp/sec (Folkow 1952; Folkow & Hamberger, 1956). Our results indicate that even small variations within this frequency range would elicit marked changes in the exchange function and cause an almost maximal constriction of the capacitance section. At discharge rates below 0.5 imp/sec major changes would occur only in the capacitance section. The marked effectiveness of low frequency stimulation on the capacitance function in the nasal mucosa as found in the present investiga-

tion, might indicate that nasal airway patency mainly is influenced by changes in sympathetic tone as previously suggested by Richerson & Seebohm (1968). The exchange function at those low frequencies, however, seems only moderately affected. To what extent this function is affected by changes in parasympathetic tone is however open to question and will be the subject of further studies.

ZUSAMMENFASSUNG

Der Effekt der sympathischen Nervaktivierung auf die Abklingquote von radioaktivem Material und das lokale Blutvolumen wurde an der Nasenschleimhaut der Katze untersucht. Es wurden die lokale Abnahme des Gehalts an ^{125}I gemessen und die Veränderungen in der Gesamtpulsfrequenzrate von ^{51}Cr markiertem Serumalbumin an der Nase registriert.

Großere Veränderungen in der Isotopen Abklingquote wurden bei einer Frequenz von 0.5-6.0 Impulsen/sec beobachtet, während ähnliche Veränderungen des lokalen Blutvolumens und Blutgehaltes bei 0.1-0.5 Impulsen/sec auftraten. Diese Befunde geben Hinweise auf die funktionelle Differenzierung der Vasokonstriktorkontrollen von Austausch- und Speichergefäßen unter niedrigem sympathischem Tonus.

Die beobachteten Reaktionen erwiesen sich als Folge einer Aktivierung von sympathischen adrenergen Nerven und Rezeptoren vom α -Typus. Für einen vasodilatatorischen Einfluss durch Stimulierung von β -Rezeptoren oder sympathischen cholinergen vasodilatatorischen Fasern ergaben sich keine Hinweise.

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AN ELECTROMYOGRAPHIC STUDY OF THE VOCAL AND CRICOTHYROID MUSCLES IN FUNCTIONAL DYSPHONIA

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Abstract. Eighteen patients with dysphonia without any signs of organic lesions as determined by phoniatric and general physical examination and consequently diagnosed as having a functional dysphonia were subjected to EMG examination of the vocal and cricothyroid muscles. In all cases indirect laryngoscopy showed the vocal cords to be symmetrical and to have a normal appearance and mobility during quiet breathing. During phonation there was an incomplete closure in the posterior part of the vocal cords in 4 patients, a minor oval shaped adduction in sufficiency ("internus paresis") in 5 patients and in the remaining 9 patients the vocal cords completely closed the rima glottidis. Motor unit action potentials were sampled from each investigated muscle by means of a transcutaneous approach and an analysis made by comparison with the muscle action potentials of healthy vocal and cricothyroid muscles. The EMG study gave conclusive evidence of neurogenic lesions in 10 cases whereas in 8 patients there were no significant deviations from normal EMG pattern. In patients with signs of neurogenic lesions EMG indicated partial paresis in most investigated muscles. In two muscles an almost complete conduction block was found. Three of the patients had an upper respiratory tract infection preceding their dysphonia, a history frequently found in idiopathic vocal cord paresis.

Among patients treated at phoniatric departments, 30-40% (Perelló 1962, Brodnitz, 1963) suffer from dysphonia in which organic lesions are not suspected to be a primary cause. These patients are commonly said to have a functional disturbance (cf Berendes, 1956, Luchsinger, 1970). The vocal cords appear normal

at laryngoscopy, i.e. there are no apparent changes in the vocal mucosa or the underlying tissue and the range and speed of the movements of the vocal cords are normal. In some cases there may be an adduction insufficiency either leaving an oval shaped minor gap between the vocal cords, or resulting in an imperfect closure of the posterior part of the glottis (Beck & Schonharl, 1959, Perelló 1962, Luchsinger, 1970).

The cause of functional dysphonia has been suggested to be a disturbance of the complex co-ordination of the different laryngeal muscles, either of habitual or psychogenic origin (Perelló, 1962). This opinion gains some support from a study of the pattern of the electromyographic activity in the vocal muscles in patients suffering from dysphonia of psychogenic origin. Thus, the activity pattern during breathing and phonation was characterised by irregularities in onset, duration and maximal amplitude (Šram & Kalvodová 1965) deviating from that seen in healthy subjects (Faaborg Andersen, 1957). Although deviations in activity pattern were observed, the action potentials did not differ in shape or amplitude from those in healthy vocal muscles, and consequently there was no support for the presence of any peripheral lesion in the nerves or muscles.

At the time when these early studies were made, the possibilities of disclosing a partial paresis in a laryngeal muscle were severely

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limited by the lack of sufficient data on the normal distributions of shapes, durations and amplitudes of the motor unit potentials in these muscles. Furthermore, the difficulties encountered in identifying a large number of potentials from a single muscle due to the continuous activity with respiratory rhythms (Weddell et al., 1944) render difficult the discrimination of a possible incidence of pathologic action potentials. By the accumulation of data on motor unit potentials in healthy vocal (Knutsson et al., 1969) and cricothyroid (Haglund, 1973) muscles from recordings by a method allowing the identification of some twenty motor unit potentials in each muscle, the possibilities of discerning even partial paresis are relatively good. This is shown in studies of traumatic (Haglund et al., 1970) and idiopathic (Haglund et al., 1972, 1973a) vocal cord pareses. Hence, this method was used in the present study to ascertain whether or not there may be any peripheral injuries in the vocal or cricothyroid muscles in patients with dysphonia where ordinary clinical examinations disclose no relevant signs of organic lesions. As will be shown, neurogenic lesions are actually frequent in such cases of dysphonia. Consequently, they are not to be regarded as functional and may in several cases be partial idiopathic pareses of muscles innervated by either or both branches of the vagus with motor fibres to the larynx.

MATERIAL AND METHOD

Eighteen patients with dysphonia were studied in which there was no indication of organic lesion as determined by phoniatric and general physical examinations. The patients included in the study were 8 men and 10 women with a mean age of 45 years (age range 17–66 years). In all cases the vocal cords were symmetrical and of normal appearance and mobility during quiet breathing as judged from indirect laryngoscopy. At phonation there was an incomplete closure in the posterior part of the vocal cords in a few patients, a minor oval

shaped adduction insufficiency ("internus paresis") in some others, and in the remainder the vocal cords completely closed the rima glottidis. This difference in appearance of the vocal cords at phonation was used to classify the investigated patients into three groups. Thus, group I consisted of 4 patients with incomplete closure of the posterior part of the glottis. This group included 1 man and 3 women (age range 41–48 years) who had suffered from hoarseness from 0.5–30 years. Group II consisted of 5 patients, 3 men and 2 women with "internus paresis" of the vocal cords. Here the age range was 49–66 years and the voice disorder was of a 4–10 year duration. In group III there were 9 patients, 5 men and 4 women (age range 17–62 years) with complete closure of the glottis at phonation. Their dysphonia had been present from 1–20 years.

In each case an abnormal fatigue and increasing dysphonia was experienced when the voice was stressed by much talking but the degree of the voice disturbance showed great individual differences. The aggravations of symptoms disappeared with rest, and the remaining disturbances were of almost constant intensity in all cases except in 4 patients belonging to group III, who at varying intervals had repeated exacerbations of more severe dysphonia for periods varying from a few weeks to 2 months.

The patients were subjected to electromyographic examination of the vocal and cricothyroid muscles. The exploration was performed by a transcutaneous approach during local anesthesia of the skin and of the subglottic mucosa. The procedure of recording from the vocal muscle has been described by Knutsson et al. (1969), and that from the cricothyroid muscle by Haglund (1973). Attempts were made to record from each investigated muscle about 20 motor unit action potentials set up spontaneously during quiet breathing. The recorded potentials were displayed on an oscilloscope and photographed for later analysis of shape, duration and amp. The a

Table 1 EMG data from 4 patients with dysphonia in group I including cases with posterior adduction insufficiency at phonation

			n	Duration of potentials mean \pm S D (ms)	High amplitude potentials (%)	Polyphasic potentials (%)	Giant potentials n	Interference	
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Normal m. vocalis ^a			469	3.76 \pm 1.01	10	0-15	0	Complete	1.5-2.5
Normal m. cricothyroid ^a			338	3.99 \pm 1.40	10	0-4	0	Complete	1.2-2.0
Case									
1	M. vocalis	Right	39	4.01 \pm 1.83	57**	21*	5	Incomplete	1.8
		Left	25	3.22 \pm 1.11*	52**	8	3	Incomplete	0.8
	M. cricothyroid	Right	24	4.45 \pm 1.77	54***	8***	8	Incomplete	1.0
		Left	24	3.65 \pm 0.86	29*	8***	0	Incomplete	1.0
2	M. vocalis	Right	26	4.75 \pm 1.16***	65***	35***	11	Complete	1.6
		Left	1*	—	—	—	—	None	—
	M. cricothyroid	Right	21	4.18 \pm 1.56	19	4	2	Complete	2.0
		Left	24	4.51 \pm 1.95	4***	21***	8	Incomplete	1.4
3	M. vocalis	Left	18	3.03 \pm 1.09*	45*	17*	3	Incomplete	1.5
	M. cricothyroid	Right	8	4.94 \pm 3.83	50***	0	2	Complete	1.0
		Left	13	3.61 \pm 1.13	23	0	1	Incomplete	1.0
4	M. vocalis	Right	22	5.90 \pm 1.39***	36	27***	4	Incomplete	1.0
		Left	45	5.28 \pm 1.12***	38	31***	8	Incomplete	1.1
	M. cricothyroid	Right	41	5.46 \pm 1.47***	34**	17***	10	Incomplete	1.1
		Left	44	4.94 \pm 1.64***	45***	30***	8	Incomplete	0.9

^a Duration, amplitude and shape of the potential: 10 ms, 300 μ V, ten phases.

Knutson et al 1969

Haglund 1973

during deglutition and phonation was recorded from several points in each muscle and its maximum amplitude measured and interference pattern assessed. A comparison was made of the data obtained by this procedure and corresponding data from normal vocal muscle (Knutson et al 1969) and normal cricothyroid muscle (Haglund 1973). When comparing the mean duration of a sample of potentials from a muscle, the incidence of potentials with high amplitude and the incidence of polyphasic potentials, a statistical analysis was made using the Student's *t* test. Extremely large polyphasic or discrete potentials with amplitudes up to 11 mV and often of long duration (5-13 ms) were observed in several muscles and they were classified as giant potentials.

Since the normal EMG of the cricothyroid muscle has not been available until recently the cricothyroid muscles in 8 early cases were not examined. In the remaining 10 cases attempts to record from the two vocal and the two cricothyroid muscles were made but the recordings from one of the vocal muscles in 5 patients and one of the cricothyroid muscles in 3 patients were discontinued. This was due to the inability in some subjects to relax sufficiently for identification in the recordings of individual motor unit potentials or to the wearing off of the anesthesia that impeded further exploration. Thus results will be given from EMG examinations of 31 vocal and 17 cricothyroid muscles in the 18 subjects.

RESULT

Group 1 Patients with posterior adduction insufficiency

Table I gives data from the electromyographic recordings in the four patients with dysphonia and incomplete closure of the posterior part of rima glottidis during phonation. It includes mean values and dispersions of the duration of the action potentials identified, incidence of potentials of high amplitude, incidence of polyphasic potentials and number of giant potentials. It also includes data on the pattern and maximal amplitude of the interference activity during deglutition and phonation. For comparison corresponding values from healthy muscles are also included in the table. The incidence of potentials of short duration used in previous reports to assess the probability of discharges of fibrillation potentials (cf Haglund et al., 1972, 1973a) is not given in the table since there was no increased incidence of short potentials of low amplitude in any of the muscles examined in the present study.

In one of the muscles, the left vocal of case 2, only one potential was observed despite careful search for activity with the electrode tip at many different sites within the muscle during repeated deglutitions and phonations that normally activate the vocal muscle. The observed potential had 10 phases and highly increased duration (10 ms). The findings indicate an almost complete block of nerve conduction to the muscle.

An interference pattern was observed at phonation or deglutition in all the other muscles recorded from. However, in the majority of muscles the pattern was incomplete and the maximal amplitude of the interference activity somewhat lower than in normal muscles. Only in three was the interference pattern judged as complete.

The mean duration of the motor unit potentials was within normal limits in 7 of the muscles. In 5 muscles it was significantly increased, and in 2 muscles it was somewhat lower than in normal muscles. The increase in

mean duration was due to an abnormal incidence of potentials of long duration among potentials of normal length so that the dispersions became larger. Contrary to previous observations (Haglund et al., 1972, 1973a) the short mean durations in two of the muscles of Table I cannot be ascribed to discharges of fibrillation potentials. The short potentials observed were of much larger amplitude than fibrillation potentials. Consequently, the low mean duration lacks diagnostic significance in these cases.

As in paretic vocal muscles there was a large proportion of potentials of high amplitude in the majority of the muscles in Table I. In large samples of potentials collected from a series of normal muscles only 10% had an amplitude exceeding 0.7 mV in the vocal muscle and 0.8 mV in the cricothyroid. In samples of 20 potentials from individual normal muscles there were great variations in the incidence of such large potentials. Calculations from previous recordings show that the incidence of potentials ≥ 0.7 mV in 19 normal vocal muscles amounts to $10 \pm 14\%$ (mean and SD). The mean incidence of potentials ≥ 0.8 mV in 17 cricothyroid muscles was $9 \pm 9\%$. Statistical analysis with Student's *t* test showed that the incidence was significantly larger in 10 of the muscles studied in this investigation with the levels of probability indicated in the table. Giant potentials were present in all except one of the muscles.

The incidence of polyphasic potentials that in healthy vocal muscles is $4 \pm 6\%$ (mean and SD) and in healthy cricothyroid muscles is $1 \pm 2\%$ was significantly increased in 10 muscles.

The deviations with respect to mean duration, incidence of high and polyphasic potentials as well as the presence of giant potentials in the muscles of Table I gives strong support for assuming neurogenic lesions in all the patients. In the right cricothyroid muscle of case 2 the observation of two typical giant potentials was the only deviation from normal. In all the other muscles more than one

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		Left	24	3.65 \pm 0.86	29*	8***	0	Incomplete	1.0
2	M. vocalis	Right	26	4.75 \pm 1.16***	65***	35***	11	Complete	1.6
		Left	1*	—	—	—	—	None	—
	M. cricothyroides	Right	21	4.18 \pm 1.56	19	4	2	Complete	2.0
		Left	24	4.51 \pm 1.95	42**	21***	8	Incomplete	1.4
3	M. vocalis	Left	18	3.03 \pm 1.09*	45*	17*	3	Incomplete	1.5
		Right	8	4.94 \pm 3.83	40***	0	2	Complete	1.0
	M. cricothyroides	Left	13	3.61 \pm 1.13	23	0	1	Incomplete	1.0
		M. vocalis	Right	22	5.90 \pm 1.39***	36	27***	4	Incomplete
M. cricothyroides	Left		45	5.25 \pm 1.12***	38	31***	8	Incomplete	1.1
	4	M. vocalis	Right	41	5.46 \pm 1.47***	34**	17***	10	Incomplete
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^a Duration, amplitude and shape of the potential: 10 ms, 300 μ V, ten phases^b Knutson et al. 1969^c Haglund 1973

during deglutition and phonation was recorded from several points in each muscle, and its maximum amplitude measured and interference pattern assessed. A comparison was made of the data obtained by this procedure and corresponding data from normal vocal muscle (Knutson et al. 1969) and normal cricothyroid muscle (Haglund, 1973). When comparing the mean duration of a sample of potentials from a muscle, the incidence of potentials with high amplitude and the incidence of polyphasic potentials, a statistical analysis was made using the Student's *t* test. Extremely large polyphasic or discrete potentials with amplitudes up to 6 mV and often of long duration, 5-13 ms, were observed in several muscles and they were classified as giant potentials.

Since the normal EMG of the cricothyroid muscle has not been available until recently, the cricothyroid muscles in 8 early cases were not examined. In the remaining 10 cases attempts to record from the two vocal and the two cricothyroid muscles were made, but the recordings from one of the vocal muscles in 5 patients and one of the cricothyroid muscles in 3 patients were discontinued. This was due to the inability in some subjects to relax sufficiently for identification in the recordings of individual motor unit potentials or to the wearing off of the anesthesia that impeded further exploration. Thus, results will be given from EMG examinations of 31 vocal and 17 cricothyroid muscles in the III subjects.

only from one vocal cord in each case. Complete interference pattern of normal amplitude was observed in the contralateral vocal muscle in both patients but this information is not given in the table.

In case 8 in the recordings from the right cricothyroid and vocal muscle there were found significantly increased incidences of potentials of high amplitude. Among these there were typical giant potentials. Also, two giant potentials were found in the left vocal muscle. In the right vocal muscle there was an increased mean duration of potentials. In none of the muscles was the interference pattern complete. In trials to activate the left cricothyroid muscle by means of repeated deglutitions and phonations, only one action potential of normal appearance was seen other than the noise recorded from distant muscles although the muscle was explored at many points. The sensitivity of the fibres to mechanical stimuli appeared to be raised since insertion activity was prominent. The findings indicate conduction block in the left cricothyroid muscle and chronic neurogenic lesions in the other three muscles.

In case 9 there was an increased incidence of high amplitude potentials in both vocal muscles and giant potentials were recorded from all three investigated muscles. A significantly increased mean duration of potentials was found in the left vocal and in the right cricothyroid muscle. In all three muscles the interference pattern was incomplete. Thus, in all the examined muscles in case 9, signs of a chronic neurogenic lesion were present.

In one of the patients of group II, the dysphonia was preceded by an upper respiratory tract infection and developed very suddenly within a matter of hours. In the other patients the dysphonia developed without any concomitant symptoms. Fatigue after talking and hoarseness were the main symptoms and they had been of relatively constant severity in all cases after the onset of the disturbances which at the time of the EMG examinations had been present for 4-10 years.

Group III Patients with complete closure of the glottis

Table III shows electromyographic findings in the 9 patients (cases 10-18) with dysphonia in whom the vocal cords were completely closed during phonations. In this group of patients the interference pattern during deglutition and phonation was complete in all the muscles examined in 8 of the patients and an incomplete interference pattern being observed in only three of the subjects.

In 2 patients (cases 15 and 16) the mean duration of the motor unit potentials in the studied muscles were within normal limits. In the right vocal muscle of case 15 there was an almost significant increase of the incidence of polyphasic potentials, but since no other deviations were observed this fact lacks diagnostic validity.

In three other subjects (cases 10, 11 and 14) the mean durations in one of the vocal muscles in each case were somewhat lower than in healthy vocal muscles ($P < 0.01$). In all these vocal muscles, the short potentials observed had varying amplitudes within the range characteristic for motor unit potentials in normal vocal muscles. Consequently, the low mean durations was not due to presence of fibrillation potentials among the action potentials recorded. In these subjects an almost significant increase in the incidence of polyphasic potentials was observed in two muscles but neither this nor the low mean durations give any conclusive support for peripheral affections in the muscles that may explain the voice disturbances.

In the remaining 4 patients (case 12, 13, 17 and 18), the EMG findings indicated neurogenic lesions. Thus in case 12 in whom recordings were obtained from only one vocal muscle, the mean duration and dispersion of durations of the motor unit potentials were increased. The incidence of polyphasic potentials was significantly raised and among these polyphasic potentials several were of long duration or high amplitude, one having the

Table III EMG data from 9 patients with dysphonia in group III including cases with complete closure of the vocal cords at phonation

Data given as in Table I

				Duration of potentials mean \pm S.D. (ms)	High amplitude potentials (%)	Poly- phasic potentials (%)	Giant potentials n	Interference		
n								Pattern	Max amp (mV)	
Normal m. vocalis				469	3.76 \pm 1.01	10	0-15	0	Complete	1.5-2.5
Normal m. cricothyroides				338	3.99 \pm 1.40	10	0-4	0	Complete	1.2-2.0
Case										
10	M. vocalis	Right	5	3.18 \pm 0.60	0	20*	0	Complete	2.0	
		Left	25	2.93 \pm 1.15**	24	16	0	Complete	2.0	
11	M. vocalis	Right	18	2.96 \pm 1.59**	22	17*	0	Complete	1.6	
		Left	7	3.32 \pm 1.26	0	0	0	Complete	2.0	
12	M. vocalis	Right	18	4.42 \pm 1.36*	22	45***	1	Complete	2.0	
13	M. vocalis	Right	16	3.86 \pm 1.03	5	0	0	Complete	1.0	
		Left	19	3.84 \pm 1.26	41*	0	0	Complete	1.1	
	M. crico- thyroides	Right	20	4.85 \pm 1.57*	45***	30***	6	Incomplete	1.0	
		Left	26	3.84 \pm 1.61	50***	19***	8	Incomplete	1.2	
14	M. vocalis	Right	19	3.00 \pm 0.81**	5	5	0	Complete	1.4	
		Left	9	3.18 \pm 0.91	0	10	0	Complete	1.5	
15	M. vocalis	Right	24	3.65 \pm 1.09	4	21*	0	Complete	1.6	
		Left	35	3.79 \pm 1.17	3	3	0	Complete	2.5	
16	M. vocalis	Left	20	3.64 \pm 0.88	25	10	0	Complete	0.8	
17	M. vocalis	Right	15	3.68 \pm 1.91	13	33***	2	Complete	0.2	
		Left	21	4.68 \pm 2.68**	33	33***	3	Complete	0.8	
	M. crico- thyroides	Left	9	6.38 \pm 3.34**	45***	0	4	Incomplete	1.2	
18	M. vocalis	Right	49	3.92 \pm 2.07	45*	25**	8	Complete	1.6	
		Left	20	4.49 \pm 1.28*	55**	15	1	Complete	2.4	
	M. crico- thyroides	Right	16	5.16 \pm 1.72*	0	6**	0	Incomplete	1.0	
		Left	20	5.41 \pm 1.80***	30*	15***	4	Incomplete	1.6	

characteristics of a typical polyphasic giant potential

In case 13, the EMG from the two vocal muscles was normal: the almost significant increase of potentials of amplitudes >0.7 mV in one vocal muscle and the somewhat low maximal amplitudes of the interference pattern in the two muscles may well be due to random factors. From the two cricothyroid muscles, on the contrary, several findings strongly indicate chronic neurogenic lesions in these muscles. Thus, means and/or dispersions of the durations of action potentials were increased, and there was an increased incidence of potentials of large amplitude of polyphasic potentials and of giant potentials that were highly significant. The electromyographic

findings in this patient has been described recently in a case report (Haglund et al, 1973b).

In cases 17 and 18 the electromyographic activity was quite similar in the two patients. An incomplete interference pattern was seen in the three cricothyroid muscles examined and in these the mean durations of the motor unit potentials were significantly increased due to increased proportions of long potentials that were of high amplitude and/or had more than four phases. Also in the vocal muscles in these patients, the incidence of long potentials was increased as seen by the increased mean durations in two of the vocal muscles and by the large standard deviations in the two others. Polyphasicity was prominent in three of the

vocal muscles and in one of the patients the incidence of high voltage motor units was also significantly raised. Giant potentials of extremely high amplitude, some with complex wave forms and long durations were recorded from both vocal and cricothyroid muscles in both cases. The findings give conclusive support for assuming chronic neurogenic lesions involving muscles innervated by both the superior and the recurrent laryngeal nerves in these 2 patients.

The dysphonias had relatively constant intensities in 5 of the patients in this group (cases 10-14). In the others (cases 15-18) the voice disturbances had intermittent courses with periods of more severe symptoms that lasted for some weeks or months. Any infections in connection with the onset of the dysphonia were not recorded, neither were any other concomitant symptoms with the exception of a congenital abnormality in one patient (case 13). She was born with a cleft lip and her voice had been judged as dysphonic from the very start of her talking. In addition to fatigue after talking and hoarseness, present in all the patients, her dysphonia was characterised by an inability to raise the pitch of her voice.

DISCUSSION

The results of the present study indicate that neurogenic lesions in laryngeal muscles may be frequent in dysphonia diagnosed as functional from thorough phoniatric and general physical examination. In group I, including patients with a posterior adduction insufficiency of the vocal cords, signs of neurogenic lesions were present in all four cases. Among the patients with 'internus paresis', group II the larynx EMG was normal in 3 cases and showed signs of neurogenic lesions in 2 cases. In group III, including 9 patients with complete closure of the vocal cords, no significant deviations in the EMG were observed in 5 patients and conclusive signs of neurogenic lesions were found in 4 of the patients.

In the patients with posterior adduction in

sufficiency the vocal and cricothyroid muscles on both sides were examined and signs of neurogenic lesions were found in all four muscles of each patient. In group II and III all these four muscles were examined only in three of the patients. Signs of neurogenic lesions were found in all four muscles in 2 of the cases but in the third such signs were present only in two of the four examined muscles while in the other two the EMG was normal. Thus, in some cases the injury may be restricted to some of the muscles. In several of the patients in group II and III without EMG changes, all muscles were not examined and the possibility must be taken into consideration that lesions might have been present in some of the muscles not subjected to examination. Consequently, the frequency of nerve injuries in patients with functional dysphonia may be still larger than disclosed in the present study although the findings, of course, do not rule out the possibility that the dysphonia in some patients has a functional origin.

Deviations in the pattern of activation during phonation as observed in patients with dysphonia (Šram & Kalvodová, 1965) may be caused by disturbances that do not have any easily defined organic origin and thus may be regarded as functional. Such deviations might, however, also be due to lesions of the laryngeal nerves, since the demands of recruiting motor units in a muscle subjected to partial paresis will be profoundly altered.

In the muscles with neurogenic lesions normal motor unit potentials were found and in many of these muscles the interference pattern was either complete or its maximal amplitude of the same magnitude as in normal muscles. The paresis was thus only partial in these muscles. This might explain why the position and the mobility of the vocal cords in these patients appear normal or only slightly abnormal. However, in the left vocal muscle in case 2 and in the left cricothyroid muscle of case 8 there was evidence of an almost complete conduction block. In spite of this, the position and the mobility of the vocal cords

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findings in this patient has been described recently in a case report (Haglund et al., 1973b).

In cases 17 and 18 the electromyographic activity was quite similar in the two patients. An incomplete interference pattern was seen in the three cricothyroid muscles examined and in these the mean durations of the motor unit potentials were significantly increased due to increased proportions of long potentials that were of high amplitude and/or had more than four phases. Also in the vocal muscles in these patients, the incidence of long potentials was increased as seen by the increased mean durations in two of the vocal muscles and by the large standard deviations in the two others. Polyphasicity was prominent in three of the

formte Adduktionsinsuffizienz ("Internusparese") bei 5 Patienten bei den übrigen 11 Patienten kam es zu einem vollständigen Stimmbandschluss der Stimmritze. Potentiale motorischer Einheiten wurden von jedem untersuchten Muskel transkutan abgenommen und mit den Aktionspotentialen der gesunden Mm. vocales und cricothyreoideus verglichen. Die EMG Untersuchungen ergaben einen schlüssigen Beweis für das Vorliegen einer neurogenen Läsion in 10 Fällen und in 8 Fällen keine oder nur geringfügige Abweichungen vom normalen EMG Muster. Bei den Patienten mit Zeichen einer neurogenen Läsion ergab das EMG Hinweise auf eine partielle Parese der meisten untersuchten Muskeln. An 2 Muskeln wurde ein nahezu vollständiger Fortleitungsblock gefunden wie bei den idiopathischen Stimmbandparese häufig zu beobachten hatten einige Patienten (3) vor dem Auftreten der Dysphonie einen Infekt der oberen Luftwege durchgemacht.

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IMMUNOLOGICAL FUNCTION OF HUMAN TONSILS, WITH SPECIAL REFERENCE TO E- AND EAC-BINDING LYMPHOCYTES

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Abstract This investigation was carried out to determine the immunological function of the human tonsil especially the role of lymphocytes. The rosette forming technique using sheep red blood cells and complement was used. Two types of E- and EAC binding lymphocytes were found. Whereas the pattern of EAC binding lymphocytes in human tonsil was similar to that in the peripheral blood, the percentage of E binding lymphocytes was less. The percentages of rosette forming cells in E- or EAC suspensions appeared to be independent of age or sex. It was suggested that the human tonsil maintains a lifetime function through an immunocompetent system.

It is well known that tonsils play a certain role in the immunocompetent system, although there are inconsistencies among the results of the different authors (Tabata et al., 1967, Surjan et al., 1968, 1972, Godrick & Patt, 1971).

Recently, an advance in immunological techniques (Brain et al., 1970, Lay et al., 1971) demonstrated that lymphocytes from man and primates showed a high proportion of cells adhering to sheep erythrocytes (E), which formed rosettes. It is now understood that certain lymphocytes are characterized by membrane markers or receptors for the binding of sheep erythrocytes. It has also been confirmed that the lymphocytes binding with E are equivalent to a thymus-dependent or derived cell (T).

Bianco et al. (1970) described a population of lymphoid cells from animal species, including man, which were identified through a

membrane receptor characteristically binding with E, sensitized by antibody-complement. These cells are designated EAC-binding lymphocyte, and their distribution corresponds to mouse "B" cells. The present investigation was carried out to determine the following: (a) the subpopulation of E- and EAC binding lymphocytes in human tonsils, (b) a comparison of E- and EAC-binding lymphocytes in peripheral blood and tonsil, (c) effect on the subpopulation of tonsil cells by age and sex.

MATERIAL AND METHODS

Preparation of lymphocyte suspension from peripheral blood and tonsil

Heparinized peripheral blood and cell suspensions obtained from minced palatine tonsils were gently placed on the surface of 3 ml of 6% Ficoll with 12% Anglo-Conray solution. The test tube was then centrifuged at 1500 rpm for 10 min. The lymphocyte rich layer visible in the tube was removed by micropipette and washed with phosphate buffered saline (PBS) as shown on Fig. 1. This suspension then contained 90% of lymphocytes.

Sheep erythrocytes (E)

Sheep erythrocytes in Alsever's solution were washed thoroughly in PBS at pH 7.4 and diluted to appropriate suspension of 0.5%.

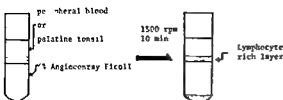


Fig 1 Schematic drawing and photograph of lymphocyte suspension

Rabbit anti E anti sera (A)

The antisera as amboceptor was prepared in rabbit using boiled stroma of E, and 1:250 dilution of antisera was used for EAC reaction.

Detection of receptor in cell suspension

For detection of E receptor, lymphocyte suspension of 0.25 ml of 4×10^6 /ml adjusted was mixed with an equal volume of 0.5% E in PBS (pH 7.4) in a test tube and incubated under appropriate condition at 37°C for 5 min. The treated mixed cell suspension was gently centrifuged for 5 min at 1000 rpm and incubated

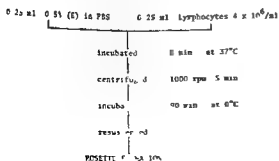


Fig 2 Detection of E receptor on lymphocytes

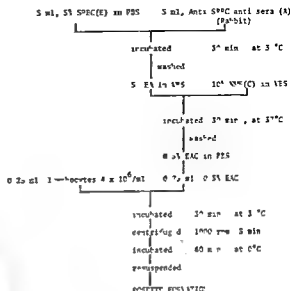


Fig 3 General procedure of detection of EAC receptor on lymphocytes

at 0°C for 90 min. The cells were then resuspended. The rosette-forming cells were examined by microscope. Detection of EAC receptor was carried out by initially incubating a mixture of 5 ml of 5% E in PBS and 5 ml of anti E sera (A) in rabbit at 37°C for 30 min. The sensitized cells (EA) were washed and adjusted to a 5% suspension in Veronal-buffered saline (VBS) at pH 7.4. Equal volume of 5% EA and of normal mouse sera (AKR strain, C) diluted 10% in VBS were mixed. The mixture was incubated at 37°C for 30 min and washed gently. EAC 0.5% suspension was prepared in PBS at pH 7.4. For the second step, equal volumes of 0.25 ml of 0.5% of EAC and lymphocytes 4×10^6 /ml were mixed. Following incubation at 37°C for 30 min, the mixture was gently centrifuged at 1000 rpm for 5 min and then incubated at 0°C for 60 min. The cell pellet was resuspended and rosette formation was observed under the microscope.

The ability to form rosettes with both E and EAC suspension was also tested. EAC suspension and lymphocytes were incubated at 37°C for 30 min, then centrifuged. Supernatant was withdrawn. Cell pellet was resus-

pended and the mixture containing 0.5% E suspension was incubated again at 37°C for 5 min and then centrifuged. Final incubation at 0°C for 1 hour was performed and the rosette was examined. The percentage of rosette forming cells was determined by microscopic observation of 400 lymphocytes. A general outline of the procedure is demonstrated in Figs 1-4.

RESULTS

Microscopic features of rosette forming cells are shown on Fig 5. As is seen in Table I the percentage of lymphocytes from peripheral blood demonstrating rosette forming ability with E suspension was 36.9 by mean values. Rosette formation with EAC suspension varied from 25.7 to 47.3% (mean value 40.9%). The ability of cell suspension from palatine tonsil to adhere to E and EAC cells is shown on Table II. The percentage of lymphocytes to bind E was 22.6 less than that of peripheral blood whereas EAC suspension represented a mostly similar pattern. Similar studies were done to determine the sex or age difference of either E or EAC binding capacity of cell suspension from tonsil.

The results are summarized in Tables III and IV. Since they demonstrate almost identical patterns, it is suggested that the percent

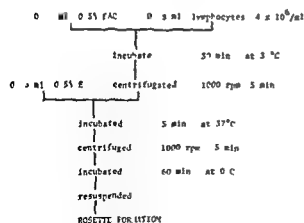


Fig 4 Detection of both E and EAC receptors on lymphocytes



Fig 5 Microscopic features of rosette forming cells of tonsil

ages of rosette forming cells in E or EAC suspension are independent of sex or age.

An additional experiment to determine precisely if the cells forming rosettes were from an E- or EAC binding cell population revealed that the percentages of binding cells with lymphocytes were incubated together with both E and EAC, corresponded mostly to the sum percentages of binding cells to either E or EAC (Table V).

DISCUSSION

Many convincing reports dealing with immunological problems of rabbit tonsils are found.

Table I Percentages of rosette forming cells peripheral blood with E and EAC suspensions

Case	E (%)	EAC (%)
1	45.2	38.4
2	40.6	34.4
3	44.7	35.7
4	44.4	33.8
5	41.0	40.8
6	39.5	45.0
7	28.3	43.5
8	50.0	45.9
9	49.5	47.3
10	47.5	41.8
11	48.2	43.7
12	43.0	45.2
13	37.2	42.7
14	30.8	39.0
15	31.6	45.3
16	38.4	36.1
17	28.6	46.2
Mean \pm S.D.	36.9 \pm 6.9	40.9 \pm 5.6

Table II Percentages of rosette-forming cells of tonsil

Case	E (%)	EAC (%)
1	21.3	29.3
2	27.4	35.1
3	21.2	29.7
4	25.1	29.5
5	28.5	63.9
6	22.3	59.8
7	15.3	37.3
8	20.0	27.3
9	24.3	36.9
10	20.5	38.7
Mean \pm SD	22.6 \pm 3.7	38.7 \pm 12.2

Table IV Age differences in the percentage of rosette-forming cells with E and EAC suspensions

Case	Age	E (%)	EAC (%)
4	11	25.1	29.5
5	5	28.5	63.9
7	3	15.3	37.3
11	3	13.5	39.5
Mean		20.6	42.5
1	23	21.3	29.3
2	37	27.4	35.2
3	51	21.2	29.7
6	26	22.3	59.8
8	20	20.0	27.3
9	20	24.3	36.9
10	44	20.5	38.7
Mean		22.4	36.7

in the literature, yet the real functions are still unknown. In our earlier and more recent experiments (Tabata et al, 1967, Okamoto, 1972a, b, Tabata et al, 1973), it was proved that the lymphocytes of rabbit tonsil have a mainly ARC-like character on immunological response, and are derived from bone marrow. Surjan et al (1972) suggested that rabbit tonsils take part in the immunological process as non regional lymph nodes, playing a role not only as antigen messenger from regional lymph nodes but also in antigen uptake. In contrast to the large number of experiments dealing with the immunological function of the rabbit tonsil, there are few reports on human tonsil because of the difficulties of experimental techniques.

Recent advance in immunocytology (Bianco et al, 1970; Lay & Nussenzeig, 1968; Lay et al, 1971) demonstrates that there are two lymphocyte populations in peripheral blood

and in lymphoid organ, either of which bind E or EAC.

It has also been confirmed that E binding lymphocytes are equivalent to T cell and the others to B cell. We were therefore interested to use this technique on the lymphocytes from human tonsil.

The present experiment suggests that the human tonsil can supply two different kinds of lymphocytes having immunological properties, such as the thymus or bone marrow derived cells in mice. It should be stressed that this cellular pattern of the human tonsil is apparently different from that of rabbit, as previously described by Richter and co-workers (1970) and Tabata et al (1973). It should also be mentioned here that in cells binding with E suspension there is a slight difference in the percentages between peripheral blood and tonsil due to a selective function of post-capillary venules, suggesting the presence of a thymus-dependent area. In 1972, Silveira et al re-

Table III Sex differences in the rosette forming abilities with E and EAC suspensions

Case	Sex	E (%)	EAC (%)	Case	Sex	E (%)	EAC (%)
1	♂	21.3	29.3	3	♀	21.2	29.7
2	♂	27.4	35.2	5	♀	28.5	63.9
4	♂	25.1	29.5	8	♀	20.0	27.3
6	♂	22.3	59.8	9	♀	24.3	36.9
7	♂	15.3	37.3	10	♀	20.5	38.7
Mean		22.3	38.2	Mean		22.9	39.3

Table V Percentages of rosette forming lymphocytes in tonsils with either or both E and EAC suspensions

	E	EAC	E + EAC
Mean (%)	23.1	31.9	47.3

Table VI Percentage of lymphoid organ cell suspensions forming rosettes with either E or EAC (Silveira et al., 1972)

Suspension tested	Mean % of rosettes	
	0.5% E	0.5% EAC
Lymph node	11	21
Thymus	98	0
Spleen	20	31
Thoracic duct	28	41

ported that the distribution of the lymphocytes binding with either E or EAC corresponded to thymus-dependent and independent areas in lymphoid organs. The percentages of rosette-forming cells with E and EAC suspensions are compared with Silveira's results (Table VI). It is an interesting fact that a pattern of the proportion of the rosette-forming cells in tonsil coincides with that in the thoracic duct. Recently, Zucker-Franklin & Berney (1972) selected the surface immunoglobulin bearing cells from human tonsils by reverse immunocyto-adherence technique and pronounced that the distribution of these cells in tonsil was similar to thymus.

Absence of sex or age differences in the binding ability of lymphocytes from tonsil with E and EAC cells means that the human tonsil can function throughout life in an immunocompetent system though further investigation on new born specimens should be carried out.

ZUSAMMENFASSUNG

Bei einem Versuch die rosettenbildenden Fähigkeiten der lymphatischen Zellen in den menschlichen Tonsillen zu bestimmen zeigte sich, daß es zwei mit E und EAC gebundene typische Zellen gibt. Das prozentuale Verhältnis der mit EAC gebundenen Zellen in den Tonsillen entspricht dem des peripheralen Bluts, dagegen zeigten die mit E gebundenen Zellen in den Tonsillen eine abweichenden prozentuale Verteilung. Weder Geschlecht noch Lebensalter veränderten das prozentuale Verhältnis der Rosettenbildungsfähigkeit mit E und EAC. Daraus ergibt sich, daß

die Lymphozyten in den menschlichen Tonsillen zum immunokompetenten System sehr beeinflussen.

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CLINICAL AND ENDOCRINOLOGICAL EFFECTS OF CORIOLIS ACCELERATIONS AND THEIR BEHAVIOR UNDER DRUG TREATMENT

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Abstract We have exposed 44 normal human subjects divided into 6 groups, to Coriolis accelerations reproduced in our laboratories. The subjects in 3 groups were tested after injection of placebo and those in the other 3 groups after injection of diazepam. The clinical and emotional behaviour, nystagmus, urinary 17KS, 17OHCS and CA were determined. In all subjects exposed to Coriolis accelerations following placebo injection, nystagmus, nausea, dizziness and a significant rise in urinary CA were encountered. Urinary 17KS and 17OHCS were not noticeably modified. The subjects tested after intravenous injection of diazepam tolerated the same disturbances with minor emotional reactivity: nystagmus, urinary 17KS and 17OHCS were almost unchanged and the urinary output of CA did not show any statistically significant variation. Consequently the authors came to the conclusion that for normal subjects exposure to Coriolis accelerations represents a stress which affects the adrenergic but not the hypophyseal-cortical system. This stress is consistently related to the emotional tension originated by the test itself. The possibility of preventing the adrenergic hyperactivity of emotional origin by diazepam is discussed.

Coriolis accelerations have reached a remarkable importance in everyday practice of aeronautic and space medicine. These accelerations appear in subjects who make head movements during a body rotation at constant angular speed by provoking a flow of endolymph in the semicircular canals; they arouse the excitation of the labyrinth receptors (Schubert 1931, Arslan, 1960, 1961, Bornschein, 1962). A series of clinical disturbances follows, of both subjective (nausea and dizziness) and objective nature (pallor, sweat, vomit and variations in the pulse rate and arterial pressure): these disturbances

are called "Coriolis phenomena" (Arslan, 1959, Bornschein & Schubert, 1962).

When Coriolis accelerations occur during acrobatic flights (turns, "tonneau", etc) or space missions, the psychophysical efficiency of pilots and astronauts is restricted (Caporale, 1965). The same troubles have been aroused by head movements in some cosmonauts during their orbital flights (Bergstedt, 1966).

We have examined the relationship between labyrinth excitation and both the adrenergic and the hypophyseal-cortical systems in order to reach an objective evaluation of the individual reactivity to the occurrence of Coriolis accelerations. At the same time we have studied the possibility of modifying this reactivity by drugs.

This research has been made by determining the behaviour of clinical and emotional disturbances, of nystagmus and of urinary catecholamines (CA), 17-ketosteroids (17KS) and 17-Hydroxycorticoids (17OHCS) in groups of subjects affected by Coriolis accelerations after the administration of either placebo or a drug apt to modify the emotional behaviour such as benzodiazepine.

MATERIAL AND METHODS

Studies were performed in 44 normal human subjects (20 men and 14 women) divided into six groups. Average age was 29 years (range 17 to 45 years).

In our laboratories Coriolis accelerations have been reproduced as follows the blindfolded subjects were subjected to angular rotation at constant speed whilst sitting on a rotation chair whose rotatory axis passed through the centre of his head, to this rotating motion was associated the additional movements which shifted the head from the original position with respect to the rotatory axis. We applied a positive angular acceleration of $0.5^\circ/\text{sec}^2$ until we reached a constant angular speed of $50^\circ/\text{sec}$. At this point, we varied the inclination of the chair-back of 45° (backwards) for 3 seconds. Three groups were tested after injection of placebo and three groups of an equal number of subjects were tested after injection of benzodiazepine.

We have tested various groups of subjects, since the repeated exposure to Coriolis accelerations can diminish or increase the emotional reactivity. The nystagmus has been registered by an electronystagmograph incorporated in the rotating chair. The urinary 17KS and 17OHCS, collected respectively 4 and 24 hours before and after the experiment, were determined one with the method of Porter & Silber and the other with the method of Collow & Zimmerman. Catecholamines (A and NA) were detected in the urine collected 1 hour before and 1 hour after the test, with the method of von Euler (D'Amelio & Iton, 1966). Placebo and benzodiazepine¹ were administered in the brachial vein 15 minutes before the test. The placebo consisted of a solution of water and 5% of glucose.

RESULTS AND DISCUSSION

In all subjects exposed to Coriolis accelerations, after a placebo administration, the Coriolis phenomena have been encountered: nystagmus, nausea, dizziness etc. The urinary output of 17KS and 17OHCS did not undergo any significant modification. Urinary CA was increased in all subjects and the variations were statistically significant. It seems evident to us therefore, that in all subjects Coriolis accelerations have

¹ 7-chloro-1,3-dihydro-1-methyl-5-fenil-2H-1,4-benzodiazepine-2-one (Diazepam) at dose of 70 $\mu\text{g}/\text{kg}$.

Table I

Urinary catecholamines (nanograms/m ²)			
No of Cases	Base	After stimulus	P
<i>Subjects without drug treatment</i>			
7	20.40 ± 5.77	30.92 ± 6.49	<0.01
<i>Subject under drug treatment</i>			
7	23.34 ± 6.67	29.56 ± 7.83	n.s.

stimulated the labyrinth receptors and have represented a stress affecting the adrenergic system but not the hypophyseal-cortical system. This behaviour is not surprising: the stress can act mostly or exclusively on one rather than the other system, as has been reviewed by von Euler already in 1964.

The reasons which may have determined a rise in urinary output of CA in subjects tested after iv injection of placebo are various. The secretion rate of NA and mostly of A is increased under emotional and nervous strain (Euler, 1964). Besides, an enhanced activity of the sympathetic nervous system can be the counterpart of the enhanced tone of the parasympathetic nervous system (Arslan & Porta, 1947).

The subjects tested after intravenous injection of benzodiazepine, have shown, to a lesser degree, the same Coriolis phenomena, but it is very interesting to remark that they have tolerated the same disturbances with a minor emotional reactivity. The nystagmus has appeared almost unchanged, urinary output of 17KS and 17OHCS

Table II

No of cases	17 Ketosteroids (urine, mg 4 hours)			17 Hydrocorticoids (urine, mg 4 hours)		
	Base	After stimulus	P	Base	After stimulus	P
<i>Subject without drug treatment</i>						
5	2.05 \pm 0.48	2.13 \pm 0.96	n.s.	1.37 \pm 0.36	1.49 \pm 0.53	n.s.
<i>Subjects under drug treatment</i>						
5	3.02 \pm 0.64	2.60 \pm 0.58	n.s.	2.61 \pm 0.45	2.32 \pm 0.61	n.s.

Table III

No of cases	17 Ketosteroids (urine, mg/24 hours)			17 Hydroxycorticoids (urine, mg/24 hours)		
	Base	After stimulus	P	Base	After stimulus	P
<i>Subjects without drug treatment</i>						
10	11.50 ±2.64	10.87 ±4.48	n.s.	8.87 ±2.03	9.42 ±2.45	n.s.
<i>Subjects under drug treatment</i>						
10	8.09 ±3.24	9.54 ±4.14	n.s.	10.01 ±1.82	9.61 ±1.97	n.s.

(time as above) has not been modified, and urinary output of CA has been enhanced although with lower and not statistically significant variations.

One other point remains to be discussed whether the decreased elimination of CA present in all subjects treated with this drug can really be connected with the action of the drug itself. This hypothesis is supported by the following considerations: Experimental physiological research has proved that diazepam diminishes the excitability of the amygdala and reduces the duration of afterdischarge of the hippocampus, a part of the limbic system which regulates the emotional behaviour (Himwich et al, 1962, Morillo, 1962, Schallek et al, 1964). The activity of the limbic system is strictly connected with the functionality of the hypothalamus, where we find located those sympathetic nervous system centres which activate the processes of integration of the autonomous visceral muscular system in order to mobilize the organic resources in a situation of stress (Arrigo et al, 1965, Himwich et al, 1962).

During some clinical researches, where the individual reactivity to stress has been determined with different parameters such as the modification of the galvanic resistance of the skin and the variation of the respiratory and cardiac frequency it has been proved, in agreement with us, that diazepam decreases the reactivity of individuals to stress (Clemens & Selesnick, 1967).

It remains to be established on which of the mechanisms originating a situation of stress

after exposure to Coriolis accelerations, the drug can be effective. Any interference with the labyrinthine receptors of stimulation may be excluded since the Coriolis phenomena have also appeared in subjects treated pharmacologically, in particular, nystagmus has always been present. The clinical observation of lower emotivity, followed by a lower increment of CA, in subjects treated with diazepam seems rather to prove that this drug—given the functional mechanism described above—acts on the emotional component of stress. An investigation on pilots trained for parabolic flights subjects to gravitation is in agreement with this hypothesis. In these subjects, exposure to Coriolis accelerations brings on the disturbances originated by labyrinth receptor stimulation but not the hypersecretion of CA (Colehour, 1964). This behaviour is attributed by the author to the elimination of emotional tension and fear through training. In the final result the situation is analogous to that obtained with the administration of diazepam.

The experiments of other authors have shown that in the normal subjects emotional stress experimentally induced (film projection) was accompanied by a significantly increased excretion of urinary catecholamines, in three patients with bilateral labyrinthine destruction the adrenal excretion remained very low throughout the experiment and the emotional stress was not accompanied by the rise found in the healthy subjects. These results are very interesting but it is very difficult to compare them with those obtained by us owing to the different experimental conditions (Fluur et al, 1967).

Thus we came to the conclusion that the exposure to Coriolis accelerations represents a stress for a normal subject. This stress increases the activity of the adrenergic system but not of the hypophysial-cortical one, and is consistently connected with the emotional tension brought forth by the test itself. The possibility of decreasing the adrenergic hyperactivity of emotional origin by drugs (diazepam) can lend itself to useful developments.

ZUSAMMENFASSUNG

44 normale Versuchspersonen, die in 6 Untergruppen unterteilt waren, wurden im rotierenden Stuhl Coriolis-Beschleunigungen ausgesetzt, und zwar wurden diese Tests an den Versuchspersonen von drei dieser Gruppen nach Injektion mit Placebo und an den Versuchspersonen der andern drei Gruppen nach Injektion von Diazepam durchgeführt. Das klinische und emotionelle Verhalten, Nystagmus und der Gehalt an 17-Ketosteroiden (17-KS), 17-Hydroxycorticoiden (17-OHCS) und Katecholaminen (CA) im Harn wurden bestimmt.

Bei allen Versuchspersonen, die nach Placebo-Injektion Coriolis-Beschleunigungen ausgesetzt worden waren, wurde Nystagmus, Übelkeit, Schwindelgefühl und ein signifikantes Ansteigen an CA im Harn festgestellt. Dagegen zeigten 17-KS und 17-OHCS im Harn keine merkbare Änderung. Die Versuchspersonen, die nach intravenöser Injektion von Diazepam getestet wurden, tolerierten die gleichen Störungen mit geringerer emotionaler Reaktivität. Der Nystagmus und der Gehalt an 17-KS und 17-OHCS im Harn waren fast unverändert und der CA-Betrag im Harn zeigte keine statistisch signifikante Veränderung. Die Autoren kamen daher zu dem Schluss, dass die Belastung mit Coriolis-Beschleunigungen für normale Versuchspersonen einen Spannungszustand (Stress) bedeutet, der das adrenerge, aber nicht das hypophysär-cortikale System beeinflusst. Dieser Stress ist engstens verknüpft mit der emotionalen Anspannung, die durch den Test selbst ausgelöst wird. Die Möglichkeit, der adrenergen Hyperaktivität emotionalen Ursprungs durch Diazepam vorzubeugen, wird diskutiert.

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SELECTIVE HABITUATION OF VESTIBULAR NYSTAGMUS BY VISUAL STIMULATION

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Abstract The effect on vestibular nystagmus of repeated exposure to a unidirectional rotating visual field was studied in ten subjects. A visually induced sensation of self rotation (circularvection - CV) about a vertical axis was repeated 30 times for each subject. Post stimulation rotation with eyes closed showed a significant 40% reduction in vestibular nystagmus intensity in the direction of the CV, measured by cumulative displacement, and no significant reduction in the opposite direction.

Vestibular habituation, the reduced nystagmus and subjective response upon repeated vestibular stimulation, is a well known phenomenon which can be produced in the laboratory (by rotation or caloric stimulation) and which occurs normally in dancers, skaters, pilots and others experiencing repeated motion patterns. The practical protective role of habituation, in preventing the disorientation and high nystagmus level associated with strong semicircular canal stimulation, is evident. An extensive review of the many animal and human vestibular habituation studies (Collins, 1973) leads to the interpretation of vestibular habituation as the development of conditioned compensatory reactions to oppose inappropriate responses associated with visual vestibular conflicts and exposure to unusual motion environments.

Most of the habituation experiments have

shown the habituation to be quite specific to the stimulus situation, as to the direction, plane and frequency of the rotation, the orientation of the head, the visual input and the manner of stimulating the labyrinth. Repeated caloric testing, although habituating the nystagmic response to further caloric stimuli, does not generally transfer to reduce the response to rotation (Collins, 1973). Only by repeated equivalent binaural caloric stimulation with visual fixation was any transfer of habituation to a rotation test observed (Pfaltz & Piffko, 1970). There are a number of situations in which non vestibular inputs have been shown to influence vestibular responses. In addition to the important influence of arousal on vestibular nystagmus (Torok, 1970), the response is effected by visual fixation and optokinetic stimulation (Jung, 1948), and by a conditioned response using auditory stimulation (Arslan et al., 1970). Acquisition of vestibular habituation can be blocked by anesthesia, and its effectiveness reduced by adrenaline (Hood & Pfaltz, 1954) and by ingestion of alcohol (Aschan et al., 1956). Furthermore, there is indirect evidence of common central mechanisms for generation of optokinetic and vestibular nystagmus (Trincker et al., 1961; Tibbling, 1970; Fukuda & Tokita, 1964). An unanswered question was whether or not a pure visual stimulus could reduce the response to a subsequent pure vestibular stimulus, demonstrating

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a transfer of habituation across sensory modalities

Under appropriate visual conditions a large peripheral field, moving uniformly around a subject, is known to produce a sensation of self rotation (circularvection, or CV), which can be extremely powerful and nearly indistinguishable from a true body rotation (Mach 1875, Fischer & Kornmüller, 1930, Dichgans & Brandt, 1972, Young et al., 1973). Although this effect can be produced about horizontal as well as vertical axes (Dichgans et al., 1972) it is most similar to a normal vestibular rotation test sensation for rotation about the vertical. Circularvection may be produced either with or without a fixation point. Repeated exposure to a small optokinetic stimulus without a fixation point, does not reduce OKN, but rather increases it, nor does it reduce the vestibular response measured by caloric or galvanic stimuli (Miyoshi et al., 1973). Repeated optokinetic stimulation does, however, produce a directional change in the stepping test in men (Miyoshi et al., 1973) and in head nystagmus in animals (HinokI & Terayama, 1966). We created an experimental situation in which a pattern of moving stripes created a sensation of rotation which closely resembled that of the vestibular rotation tests but did not induce OKN because of a fixation point. We studied the effect of repeated visual stimuli on the response to a vestibular rotation test administered afterwards.

METHODS

Subjects

Thirteen subjects from the hospital staff were tested, none having known vestibular or neurological disorders. The three women and ten men ranged in age from 20 to 47. Three subjects were excused from further testing when the nystagmography revealed spontaneous nystagmus with eyes closed. In the remaining group some subjects showed varying degrees of direction preponderance on vestibular testing. Three other subjects were given a reversed order pre test and post test to check for the effect of test

order. The remaining seven subjects underwent the normal protocol described below.

Pre-test and post-test rotations

Before and after exposure to the visual stimuli all subjects were accelerated right and left to test the strength of vestibular nystagmus in each direction. In the normal protocol, as shown in Fig. 1a, subjects were accelerated clockwise about a vertical axis, at an acceleration of 5 deg/sec^2 for 12 sec, rotated at a constant speed of 60 deg/sec for 45 sec and finally decelerated at 5 deg/sec^2 to a stop. Motion was controlled using a Tönnies (Freiburg im Breisgau/Germany) rotating chair. All tests were performed with eyes closed in the dark. Nystagmus and subjective sensation of rotation were recorded from 30 sec prior to acceleration until 30 sec after the chair had come to a stop. For the reversed order protocol, the pre and post tests were performed with counterclockwise rotation the direction of the visual stimuli remaining unchanged. For one subject (7) all accelerations were for 15 sec, and the constant velocity period was at 75 deg/sec .

Control tests

To separate the habituating potential of the visual stimuli from any response decline resulting from the repeated rotation the pre test and post test sequence was also administered to each subject at a separate time from the habituation test. No intermediate moving stripe stimulus was given. To avoid order effects and habituation to repeated tests and to investigate the duration of any visually induced habituation the control test was given to each subject only once, either in the month before the main experiment or during the 12 months after the experiment.

Visual stimulus

The visual stimulus used to generate circularvection about a vertical axis was similar to that used in other circularvection studies (Dichgans & Brandt, 1972, Young et al., 1973). The Tönnies moving shadow optokinetic stimulus ge-

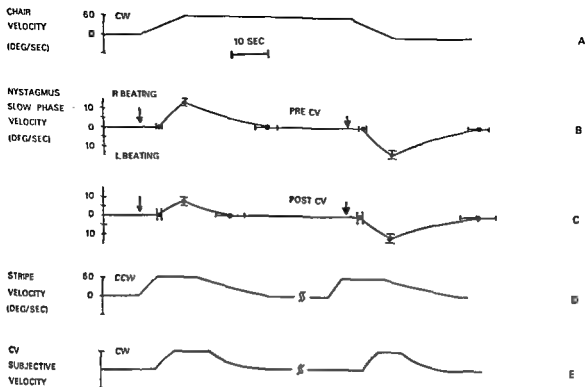


Fig 1 Average pre- and post test nystagmus responses and typical CV stimuli (A) Pre and post test chair angular velocity, normal protocol (B) Average pre-test vestibular nystagmus response, ten subjects. Circles show mean latency, mean slow phase velocity averaged over ten seconds (see methods), and mean duration. Bars show standard error of these means. Lines connecting data

points are merely suggestive of the nystagmus waveform. Arrows line up with onset of acceleration (C) Average post CV vestibular nystagmus, as in B (D) CV stimulus stripe velocity. Two representative waveforms of a series of 30 (E) Sketch of typical CV velocity estimates corresponding to the stimuli in D.

rator was modified to generate a desired circular-vection dynamic response. The subject was centered before a white screen, one meter in radius. The screen was 135 cm high and covered a visual angle approximately 31 deg above, 37 deg below, and 80 deg to each side of the eyes in primary position. The dark stripes subtended 6°40' and were separated by 8 deg. The subject held his head fixed and upright in a dental head rest, and fixated a small (19 mm × 19 mm) dark square placed in the center of the screen at eye level. The visual stimulus was a stripe motion in the counterclockwise direction, producing only clockwise subjective velocity. For each CV exposure the stripes were accelerated from rest to a speed of 60 deg/sec counterclockwise (75 deg/sec for subject 7) over a period varying from 3 to 15 sec. The constant velocity period

lasted from 10 to 60 sec, following which the stripe speed slowed exponentially toward zero velocity with a time constant of 12 sec and stopped after 15 to 30 sec. The movement pattern of the stripes (acceleration, constant velocity, and deceleration) was chosen to produce corresponding sensations of increasing, constant, and decreasing CV. It was intended to produce CV patterns which mimic the counterclockwise sensation produced by the first part of the rotation stimulus used in the pre- and post-tests. This procedure was repeated 30 times with pauses of 5–20 sec between trials.

Subject instructions and control of arousal

Subject arousal was maintained at a high level to promote nystagmus by requiring the signalling of 90 deg turn intervals (Collins & Guedry,

1962, Collins, 1964) During the pre- and post-rotation tests, subjects were instructed to close their eyes, look straight ahead, and concentrate on the signalling task. A small three position switch was used to indicate the time and direction of the onset of rotation, each 90 degree interval, and the cessation of turning. During the circularvection tests subjects were instructed to look at the fixation point and indicate any self motion in the same manner as during rotation in the dark.

Data recording and analysis

Horizontal eye movements were recorded by electronystagmography, with a 3 sec time constant. Eye movement records were hand analyzed by one of us unaware of the direction of CV for each subject. Cumulative eye displacement, used as a measure of average slow phase velocity, was measured over the peak 10 sec nystagmus activity period by taking the sum of all fast phase amplitudes. Beat frequency was calculated for this same period. Nystagmus latency was measured from the onset of acceleration or deceleration to the first fast phase, and duration was measured to the last fast phase. Subjective turning latency, peak subjective velocity and rotation were also calculated from the switch actions.

All statistical analyses were performed using T-tests on paired differences.

RESULTS

In all subjects tested vestibular nystagmus for acceleration in the direction of circularvection was reduced following exposure to the visual stimulus. The average nystagmus response, for all ten subjects is shown in Fig. 1B for the vestibular pre test and in Fig. 1C for the post test. The average latency, duration and peak slow phase velocity (as estimated from cumulative eye displacement) are indicated with the standard error of these averages. The right and left beating nystagmus of the reversed order protocol subjects were averaged with the normal protocol right and left nystagmus respectively.

All subjects experienced CV in the direction opposite to stripe motion. Typical stripe velocity wave forms are shown in Fig. 1D, with sketches of common subjective turning indications given in Fig. 1E. The subjective indication of turning usually commenced 3-10 sec following the beginning of stripe motion, continued at a relatively constant rate during constant speed stripe rotation, and declined gradually during the decay of stripe speed. No consistent pattern of change in subjective response latency or peak velocity was seen during the thirty CV trials. Because of the fixation point, no measurable optokinetic nystagmus occurred during the CV trials.

Fig. 2 shows one case of nystagmus decline. In this case an initially strong and regular right beating vestibular nystagmus (top trace) was reduced markedly in slow phase velocity, frequency and duration following exposure to thirty CV-right stimuli (second trace). The weaker initial left beating vestibular nystagmus (third trace), on the other hand showed only a slight decline following the CV stimuli (bottom trace).

Cumulative displacement

As expected, of the various nystagmus parameters measured, only cumulative displacement showed a clear and consistent direction specific response reduction. Table I lists the cumulative displacement for each subject, in each direction, pre- and post-exposure to the CV stimuli, and for the control tests. The post test cumulative displacement declines relative to the pre test level in the direction of CV, for each of the ten subjects. The average decline is 40% and is significantly different from zero ($p < 0.0025$). In the direction opposite to CV, on the other hand a response decline in cumulative displacement was only present in 7 of the ten subjects (average 16%) and was not significant at the 0.1 level. When each subject's decline is compared as to the direction of acceleration relative to CV, the declines in the direction of CV are an average of 23% larger than those for acceleration opposite to CV ($p < 0.05$). The pre- and post test

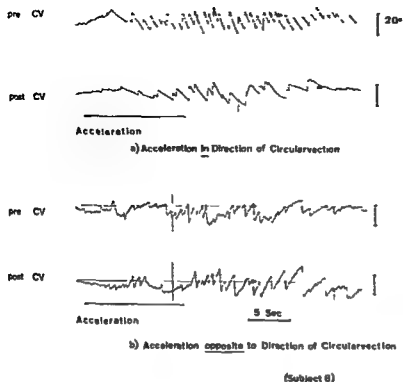


Fig 2 Vestibular nystagmus recordings from one subject before and after exposure to 30 unidirectional circularvection stimuli

displacements for each subject in the control tests, without any CV, indicate no consistent or significant response decline associated with the repeated testing

When each subject's response decline in the direction of CV is compared with his own change in response in that direction for the control test, this percentage decline is an average of 53% larger ($p < 0.0025$). Thus one effect of the repeated unidirectional CV trials is to reduce significantly the vestibular nystagmus cumulative displacement for true body acceleration in the direction of CV, relative both to control tests without CV and to accelerations in the opposite direction

Other measures

The only other nystagmus parameter found to be effected by the CV tests was the average frequency of nystagmus during the peak velocity period. Contrary to the findings of Johnson & Torok (1970) who measured vestibular nystagmus frequency with eyes open in the dark, and

the earlier findings of Wendt (1965) and Collins (1964) nystagmus frequency decreased slightly in the post-tests. The average frequency declined 23% in the CV direction ($p < 0.1$) and 21% in the direction opposite to CV ($p < 0.1$). There was no significant difference between the two directions. The frequency measures were found to be more variable and less sensitive to habituation than the measures of slow phase velocity. It is conceded that the measurement of frequency is a less reliable parameter, especially when recording conditions are noisy. To exclude such errors only fast phases with amplitudes of more than 2 deg were counted. The resolution of the recording system was better than 1 deg. Latency and duration measures did not show any significant evidence of any habituation. Subjective measures were similarly unrevealing.

Duration of habituation

Although a systematic investigation of the duration of the visually induced vestibular nystagmus habituation was not part of the

Table 1 Vestibular nystagmus cumulative displacement, in degrees, measured during the peak velocity ten second period for each acceleration

Subject	With CV stimuli				Without CV stimuli			
	Acc w CV (clockwise)		Acc opp CV (counterclockwise)		Clockwise acc		Counterclock- wise acc	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
1	57	48	37	37	106	103	98	125
2	138	46	140	202	81	102	125	101
3	68	45	57	45	170	240	167	207
4	122	16	97	8	154	132	140	182
5	137	17	136	36	138	110	137	88
6	110	85	188	130	86	122	29	51
7*	205	154	200	179	74	112	120	100
8	168	78	125	122	105	158	133	130
9	255	250	337	280	230	241	229	194
10	51	49	56	77	107	67	163	115
Average decline pre-post tests	40% ($p < 0.0025$)		16% (N.S.)		-13% (N.S.)		-2% (N.S.)	

* - tested at 75 deg/sec, data normalized to 60 deg/sec

experimental plan, incidental observations are relevant. Control rotation tests were performed on seven subjects at times ranging from two weeks to three months following the CV stimulus. In no case did any sign of the earlier habituation reappear. Furthermore, four of the subjects were given a second acceleration post test, a few minutes after the first post-test,

which immediately followed the CV stimulus series. In each of these re-tests, the habituation (primarily in the direction of the CV as seen in the first post-test) was nearly completely absent in the second test. Finally, for two of three subjects (8-10), receiving the reversed order protocol, in which they were tested in the opposite CV direction immediately after the CV series, and then in the CV direction, the reduction in the CV direction was small. All of these observations point to the conclusion that the response reduction resulting from this short, one-half hour exposure to a CV stimulus, is extremely brief compared to the long duration response reductions associated with repeated accelerations (Hood & Pfaltz, 1954; Collins, 1964). They also confirm the importance of testing order in searching for vestibular directional effects (Aschan et al., 1952).

DISCUSSION

Several lines of evidence suggest that vestibular habituation is of central rather than of peripheral origin. Some of the more persuasive experiments supporting this view come from the inhibiting effect of the efferent vestibular processes (Groen, 1965; Goetmakers, 1970), the specific habituation to certain patterns of motion (Aschan 1954; Collins, 1968), and the importance of alertness in regulating the level of nystagmus and response reduction (Collins, 1964; Guedry, 1965; Benson et al., 1966). Of particular interest is the fact that stimulation of the vestibular organ is not, in itself, sufficient to produce vestibular habituation under slightly altered circumstances. Habituation gained with vestibular stimulation in one head orientation does not transfer to an altered head position, to a different axis of rotation, or to a new direction of acceleration about the same axis (Collins, 1969; Crampton & Brown 1964; Collins, 1964). Finally, the failure to obtain transfer of habituation from one stimulus mode to another is particularly noteworthy. Thus stimulation of the vestibular apparatus has been shown not to be a sufficient condition for vestibular habituation. The current experiment

demonstrates that it is also not a necessary condition, since exposure to a purely non-vestibular input led to vestibular habituation. The term "vestibular habituation", as generally used to refer to a decline in the vestibulo-ocular response upon repeated tests is appropriately used for the phenomenon found in this experiment, even though no adequate vestibular stimulus was involved in the habituation process. On the other hand, the absence of OKN during the CV trial precludes any localization of the habituation on the motor side. The transfer of habituation across sensory modalities is a proof of its central origin.

The reason for the success of CV habituation transfer to rotation stimuli and the usual failure of caloric habituation to transfer probably is associated with the different orientation of the head (Collins, 1964, 1969). The similarity of the effects of CV stimulation to those of true rotation extend to the influence on vestibular thresholds (Young et al., 1973) and the production of the pseudo-corniolis sensation (Dichgans & Brandt, 1973). The production of nystagmus by the habituating stimulus seems unrelated to the efficacy of the stimulus in habituating vestibular nystagmus associated with rotation in the dark. Furthermore, Brandt et al. (1973) have shown the independence of CV and optokinetic nystagmus. We cannot, of course, eliminate the possibility that the stimulus is one which would tend to produce nystagmus, but that the visual fixation system counters this and that the process of actively countering nystagmus is, in itself, contributory to the habituation.

One interpretation of this experiment, in conjunction with the caloric transfer experiments, is that a habituating stimulus must produce the same subjective sensation of motion as that produced by true body motion in order to habituate vestibular nystagmus in the latter case. Stimuli which do not produce identical subjective sensations, because of conflicting visual-vestibular relationships or because of intra-labyrinthine conflicts, are not adequate to transfer nystagmus habituation between different modes of stimulation even though they may produce

similar nystagmus patterns. As Nilsson (1972) has pointed out, vestibular nystagmus and sensation of turning, although responding to the same stimuli and following similar time courses, are only weakly correlated and are regulated at two separate levels.

Speculation on the site of the visually induced habituation is somewhat premature. The finding of the current experiment, that visual inputs inhibit a vestibular response in a manner entirely similar to that of vestibular inputs, is consistent with the hypothesis that the visual input in fact biases the vestibular signal and produces, at the neural level, a change in firing pattern which is indistinguishable from that produced by pure vestibular stimulation (Klinke & Schmidt, 1970, Dichgans & Brandt, 1972, Dichgans et al., 1973, Henn et al., 1973).

ZUSAMMENFASSUNG

Der Effekt eines sich nach rechts drehenden visuellen Feldes auf vestibulären Nystagmus wurde bei 10 Versuchspersonen untersucht. 30mal wurde bei jeder Versuchsperson eine visuell induzierte Empfindung von Eigenrotation (Zirkularvektion-CV) um eine vertikale Achse hervorgerufen. Danach war der vestibuläre Nystagmus bei Drehung mit geschlossenen Augen in Richtung der CV, gemessen als kumulative Augenabweichung, um 40 % signifikant vermindert. In der Gegenrichtung war er nicht signifikant reduziert.

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INTERACTION BETWEEN THE UTRICLES AND THE VERTICAL SEMICIRCULAR CANALS

VI Unilateral Selective Sectioning of the Horizontal and Vertical Ampullar Nerves, followed by Tilting around the Longitudinal Axis

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Abstract Unilateral selective left sided sectioning of the horizontal and vertical ampullar nerves was performed. After the operation, horizontal rotatory nystagmus developed. When the cats were tilted around their longitudinal axis towards the operated ear the horizontal nystagmus increased in intensity. At the same time as the rotatory component disappeared. Tilting towards the non-operated ear increased the rotatory nystagmus and completely inhibited the horizontal component. The cause of this reaction is discussed.

Earlier investigations (Fluor & Siegborn, 1973 a b) have shown an intimate cooperation between the utricles and the horizontal semicircular canals. It has been demonstrated that one utricle, after unilateral sectioning of the horizontal ampullar nerve and the utricular nerve, during tilting of the animal around its longitudinal axis towards the operated ear, facilitates horizontal nystagmus released from the ipsilateral horizontal semicircular canal. Simultaneously, however, there also takes place an inhibition of the reflex arc from the contralateral horizontal semicircular canal (Fluor & Siegborn, 1973 c). Because electrical stimulation of a small area on the mid lateral surface of the utricle can produce horizontal nystagmus towards the stimulated ear (Fluor & Mellstrom, 1970) it can be considered as definitely established that it is this area which during tilting in the above-mentioned direction causes nystagmus. Other investigations have

shown that selective unilateral sectioning of the two vertical ampullar nerves gives rise to a counterclockwise rotatory nystagmus, with the upper pole of the eyes beating towards the intact ear. Tilting around the animals' longitudinal axis gives rise to increased nystagmus if the animals are tilted towards the intact ear, and inhibits it if they are tilted in the opposite direction (Fluor & Siegborn, 1974).

Regarding the above mentioned experiments, it may be objected that it is not possible by means of tilting to stimulate only parts of the utricular surface, since the whole surface is influenced simultaneously by the sliding of the otolith membrane. The explanations adduced for the cause of the above-mentioned reflex activity may, for the uninitiated, seem somewhat theoretical, but all the relevant facts have been stated in an earlier paper (Fluor & Mellstrom, 1970). Therefore, to make it even more clear, we intend to integrate the two experimental series with the horizontal and the vertical semicircular canals respectively and to investigate what happens when these two experiments are performed on one and the same animal.

MATERIAL AND METHOD

Eight cats were used for the experiments. They were first anesthetized with ether in a so-called

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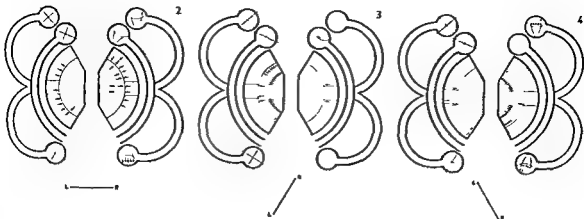


Fig 2 Diagram of the utricles and semicircular canals in resting position after unilateral selective sectioning of the left horizontal and vertical ampullar nerve. The arrows indicate the orientation of the hair cells, i.e., the direction in which they increased their discharge frequency

Fig 3 Diagram of the utricles and semicircular canals after unilateral selective sectioning of the left horizontal and vertical ampullar nerves and when the

animal is tilted toward the left. Arrow indication same as in Fig 2. The number of arrows symbolizes discharge frequency

Fig 4 Diagram of the utricles and semicircular canals after unilateral selective sectioning of the left horizontal and vertical ampullar nerves, and when the animal is tilted to right. Arrow indication same as in Fig 2. The number of arrows symbolizes discharge frequency

area of the right labyrinth and the medial area of the left labyrinth facilitating clockwise nystagmus is increased. This adequately explains the total inhibition of all counterclockwise rotatory nystagmus.

Tilting in the opposite direction has quite the contrary effect (Fig 4). Here, we have an inhibition of the activity of the mid lateral "horizontal" area on the utricular surface of the right ear, causing the inhibition of the horizontal nystagmus. The function of the areas which give rise to clockwise rotatory eye movements is on the other hand facilitated and so is the counterclockwise rotatory nystagmus.

The results obtained in the present investigation are in full agreement with clinical findings on patients with unilateral peripheral vestibular disorders described by Nylen as early as 1924.

ZUSAMMENFASSUNG

Selektive unilaterale Abschneidung der linken horizontalen und vertikalen Ampullenkanäle wurde vorgenommen. Des ergab einen horizontalen rotatorischen Nystagmus. Als die

Katzen um ihre longitudinale Achse nach der operierten Seite hin gekippt wurden wurde die Geschwindigkeit des horizontalen Nystagmus erhöht gleichzeitig verschwand die rotatorische Komponente. Eine Kippung in Richtung des nicht operierten Ohres erhöhte die Geschwindigkeit des rotatorischen Nystagmus und die horizontale Komponente verschwand. Die Gründe dafür wurden diskutiert.

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ULTRASTRUCTURE OF MIDDLE EAR MUSCLE IN THE RABBIT

II Tensor Tympani

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Abstract Two types of muscle fibers were observed. The first was of larger diameter with relatively few mitochondria and well delineated myofibrils separated by many sarcoplasmic reticulum. The myoneural junctions of these cells show well developed postsynaptic foldings. The second type was of small diameter with many mitochondria and poorly separated myofibrils with sparse sarcoplasmic reticulum. Their myoneural junctions show much less developed postsynaptic foldings.

Two types of muscle fibers have been observed in mammalian middle ear (Erulkar et al., 1964, Fernand & Hess, 1969, Hirayama & Daly, in preparation, Seiden, 1971) and extraocular (Peachey, 1971) muscles. One type is morphologically slow (Hess, 1970), the other is of the twitch type.

In rabbit stapedius muscle two varieties of twitch fibers were reported, in addition to the slow fibers (Hirayama & Daly, in preparation). Functional differences have been noted between the two middle ear muscles of rabbit. The tensor tympani has a higher reflex threshold, smaller reflex tension and longer contraction time than the stapedius (Wersall, 1959). It is thus of interest to compare these two muscles morphologically. In the present

study, the tensor tympani of rabbit was examined by light and electronmicroscopy.

METHODS

The tensor tympani muscles of normal adult albino rabbits (2.5-3 Kg) were studied in frozen sections and by electronmicroscopy. Serial frozen sections (20 μ) were incubated for succinic dehydrogenase activity (Nachlas et al., 1957) for study of the mitochondrial distributions (Gauthier & Padykula, 1966, Gauthier, 1969) within the respective fibers. Other animals were perfused with 2% glutaraldehyde and post fixed in 1% osmium tetroxide. These specimens were embedded in Epon 812 and serially sectioned by steel knife at 20 μ m for phase contrast viewing (Davidowitz et al., 1972). Such sections provided an initial light microscopic survey of the muscle whereby tentative fiber populations could be discriminated according to fiber diameter and mitochondrial content. Selected 20 μ m sections were cemented onto support blocks for further transverse sectioning at 1 μ m by glass knife to provide a more detailed view of the cells and their myoneural junctions. Correlated longitudinal and transverse ultrathin sections of the same cell were obtained from serial 20 μ m sections by a previously described

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Fig. 1 Transverse Epon section (mouse toulidine blue stain). Typical large (L) and small (S) cells are selected for further ultrathin sectioning. Myoneural junctions (arrows) are evident on both of these cells. The horizontal solid line indicates the line of match with the longitudinal section in Fig. 2. Vertical dashed lines indicate the intersection of the longitudinal and transverse cell perimeters of the large cell (L). (No inter-section with the small cell (S) is indicated insofar as the longitudinal plane of section had fallen out of that cell just before reaching this transverse plane) $\times 1200$.

Fig. 2 Longitudinal 1 μ m section cut through a 20 μ m Epon section. This is a small portion of a long ribbon of many cells which contains the pre-selected fibers L and S. The lines of perimeter match through the two transverse sections is determined by superimposing each edge of the entire ribbon over that transverse section on which had originally been contiguous with it. This 1 μ m section was taken immediately after the ultrathin sections shown in Figs 7 and 10 $\times 1200$.



Fig. 3 The second of the bracketing transverse sections. Here the solid line of match passes through the middle of the small cell (S). The upward displacement of the line of match through cells 'S' and 'L' (cf. Fig. 1) indicates a somewhat oblique plane of section through these fibers. For some of the cells appearing in Figs 1 and 3 there is much less of a shifting in relation to the respective lines of match. Such disparity of shifting reflects a considerable amount of fiber displacement in relation to each other at the middle portion of this muscle $\times 1200$.

Fig. 4 Same section as in Fig. 1 at higher magnification. Large fiber (L) shows sparse mitochondrial granularity; the small fiber (S) contains a relatively more dense mitochondrial distribution. Myoneural junctions are indicated by arrows $\times 3000$.

Fig. 5 Succinate dehydrogenase stain of 20 μ m frozen section. The large fiber (L) shows sparse stained granules; the small fibers (S) contain numerous densely packed stained granules. Presumably the granules indicate mitochondrial locations $\times 1200$.

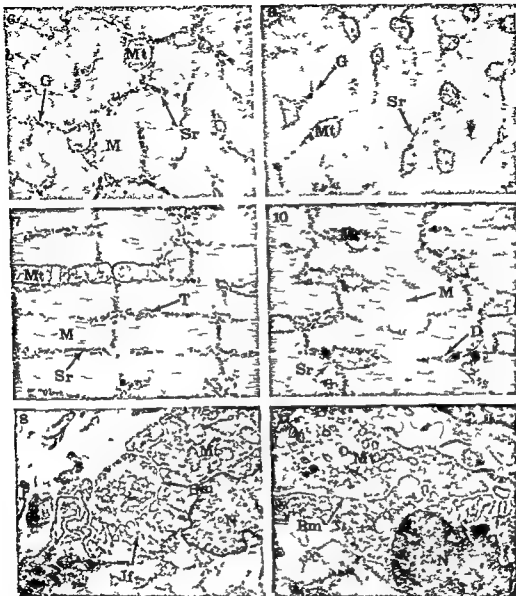


Fig 6 Transverse section of the large fiber (L) indicated in Fig 1. The myofibrils (M) are well delineated by sarcoplasmic reticulum (Sr) and some glycogen granules (G). Few mitochondria (Mt) are evident $\times 15\,000$

Fig 7 Correlated longitudinal section of the same large fiber shown in Fig 6. The myofibrils (M) are clearly distinguishable. Mitochondria (Mt) are few and the transverse tubular system (T) is frequently seen. M line. Sr Sarcoplasmic reticulum $\times 15\,000$

Fig 8 Nerve ending from same transverse section as Fig 6 showing well developed junctional foldings (Jf). Bm Basement membrane N Nucleus Mt Mitochondria $\times 10\,000$

Fig 9 Transverse section of the small fiber in

dicated in Fig 1. The myofibrils are not clearly distinguishable and blend one with the other. Mitochondria (Mt) are numerous and little sarcoplasmic reticulum (Sr) is evident. G Glycogen granules $\times 15\,000$

Fig 10 Correlated longitudinal section of the same small fiber shown in Fig 9. Myofibrils are poorly distinguishable and not well separated. Mitochondria (Mt) are abundant. Few diads (D) or triads are seen. M line (M) is occasionally discernible but indistinct. Sr Sarcoplasmic reticulum $\times 15\,000$

Fig 11 Nerve ending from same transverse section as Fig 9 showing few poorly developed junctional foldings (Jf). Bm Basement membrane N Nucleus Mt Mitochondria $\times 10\,000$

procedure (Davidowitz et al, 1972) whereby the second of three consecutive 20 μm sections is sandwiched between Epon sheets to provide a ribbon of longitudinally cut fibers (Fig 2). The first and third of the three 20 μm sections provide bracketing transverse sections through the same fiber population (Figs 1 and 3). The corresponding cell identities are established along each edge of the ribbon (Fig 2) where the longitudinal cell perimeters intersect their circular counterparts in the bracketing transverse sections along a line of match (Figs 1 and 3). By monitoring through the sandwich during the longitudinal sectioning, one may observe the transverse profile of the cells, and thus aim for preselected fibers of interest. Ultrathin sections were collected on Formvar grids (1 mm opening), stained with uranyl acetate and lead citrate, and examined by Siemens Elmiskop I.

RESULTS

Light microscopy of the 1 μm Epon sections revealed two clearly distinguishable fiber populations (Figs 1 and 4). The first was of larger diameter (30–40 μm) and contained relatively sparse mitochondria, the second was of smaller diameter (10–20 μm) and showed relatively numerous mitochondria. These two types were evidenced as well in the frozen sections treated for succinic dehydrogenase (Fig 5). Fibers of intermediate diameter and granularity were also observed. The proximal portion of the muscle is typically composed of a core of connective tissue with small cells grouped along the periphery, the distal portion of the muscle contained a mixture of the various fiber diameters.

The fine structure of a typical large diameter fiber (Fig 1 L) is shown in correlated transverse and longitudinal section in Figs 6 and 7 respectively. It contains distinct myofibrils separated by abundant sarcoplasmic reticulum. There are few mitochondria, triads are frequently observed and the M line is clearly evident. The motor nerve ending of

this cell (Fig 8) shows well developed junctional foldings, and the terminal axon lies deep within an invagination of the muscle fiber.

The typical small diameter fiber indicated (S) in Fig 1 is shown in correlated transverse and longitudinal ultrathin sections in Figs 9 and 10 respectively. Its myofibrils are indistinct, poorly organized, and appear to fuse one with the other. The sarcoplasmic reticulum is sparse, there is a moderate amount of glycogen granules, and few diads or triads are seen. The mitochondria are numerous and do not tend to form clusters or chains. An indistinct M line is occasionally discernible. The myoneural junction of this fiber (Fig 11) shows a relatively poor development of the junctional folding, and the terminal axon lies superficially on the surface of the muscle fiber. No muscle spindles were observed.

DISCUSSION

The small diameter fibers observed in this study display poorly delineated myofibrils and sparse sarcoplasmic reticulum. Such fibers have been referred to as *Felderstruktur*, and are thought to be physiologically slow (Hess, 1970). The appearance of this fiber is consistent with that of slow fibers observed in the tensor tympani of guinea pig (Seiden, 1971), and cat (Erulkar et al, 1964; Fernand & Hess, 1969), and in the stapedius of rabbit (Hirayama & Daly, 1973), and cat (Fernand & Hess, 1969). The moderate junctional folding of this fiber, however, appears to be somewhat more developed than previously indicated for slow fibers in the middle ear muscles (Fernand & Hess, 1969; Hirayama & Daly in preparation; Seiden, 1971).

The large diameter fiber observed in this study appears similar to the first type of twitch fiber (white) reported in stapedius muscle of rabbit (Hirayama & Daly, 1973), though we did not observe fibers which could be clearly classified as being of the second (red) twitch type reported in that study.

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ZUSAMMENFASSUNG

Zwei Arten von Muskelfasern wurden beobachtet. Die erste Art war von grösserem Durchmesser mit verhältnismässig wenigen Mitochondrien und deutlich ausgeprägten Myofibrillen, getrennt durch zahlreiches sarkoplasmatisches Retikulum. Die myoneuralen Verbindungen dieser Zellen haben gut entwickelte postsynaptische Falten. Die zweite Art war von kleinerem Durchmesser mit vielen Mitochondrien und kaum getrennt durch Myofibrillen mit spärlichem sarkoplasmatischem Retikulum. Ihre myoneuralen Verbindungen wiesen viel weniger entwickelte postsynaptische Falten auf.

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ULTRASTRUCTURE OF THE BASILAR PAPILLA, AN AUDITORY ORGAN IN THE BULLFROG

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Abstract The basilar papilla is one of two auditory organs found in the frog's inner ear. Examination of the papilla in the bullfrog, *Rana catesbeiana*, by light and electron microscopy has confirmed earlier descriptions of its gross morphology and revealed new details of its fine structure. The papilla is a tubular evagination of the saccule, terminating in a thin contact membrane which separates endolymphatic and perilymphatic spaces. Its sensory epithelium occupies a crest extending halfway around the tube and is surmounted by a tectorial membrane. Between 300 and 500 myelinated nerve fibers synapse with about 60 hair cells. Extending from the hair cells are sensory hair bundles, lodged in canals in the tectorial membrane. Each bundle consists of a group of stereocilia and a single asymmetrically placed kinocilium located on the side of the bundle facing the contact membrane. Each hair cell synapses at its base with several afferent terminals but efferent synapses have not been found.

The basilar papilla is one of two auditory receptor organs found within the labyrinth of anuran amphibian species (Fig. 1). Both basilar and amphibian papillae have been described in light microscopic detail by a number of investigators (van Bergeijk & Witschi, 1957; Geisler et al., 1964) and their response characteristics have been studied by electrophysiological techniques in several species (Frishkopf & Goldstein, 1963; Sachs, 1964; Capranica & Frishkopf, 1966; Frishkopf & Geisler 1966). The purpose of the present report is to describe the ultrastructure of the basilar papilla in functional

terms. A preliminary report of this work has been published elsewhere (Frishkopf & Flock, 1967).

MATERIALS AND METHODS

Young adult bullfrogs, *Rana catesbeiana*, body weight 30-100 g. were anesthetized by intramuscular injection of DIAL in urethane (CJBA), dosage 2 cc/kg. an incision was made in the skin along the dorsal midline, starting anterior and ending posterior to the external eardrum. A skin flap formed by cutting transversely at each end of the midline incision was removed close to the superior edge of the tympanic ring. The dorsal fascia was cut close to its line of medial attachment and the muscles beneath detached and retracted to expose the surface of the otic capsule. Laterally, the outlines of the anterior and posterior semicircular canals form a right angle, with the vertex directed medially.

The bone over and between the canals was thinned with a dental drill and removed by cutting with a razor or fine scissors, exposing the perilymphatic membranes. Blood flow may be observed in several small vessels in the walls of the labyrinth. An opening was made into the saccule, identified by the large white otolith it contains, through perilymphatic and endolymphatic membranes. A second opening into the endolymphatic system was made at the top of the common crus, where the vertical canals meet

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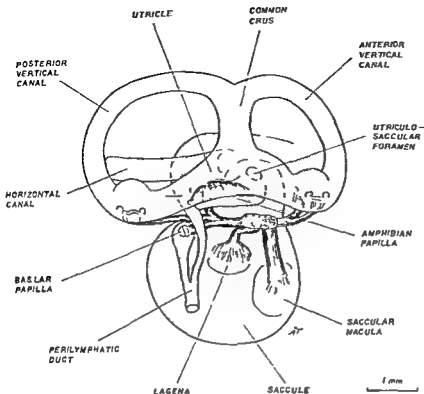


Fig 1 Schematic drawing of left labyrinth of bullfrog, from medial, showing endolymphatic system, sensory organs and their innervation. Perilymphatic duct is shown in relation to auditory papillae. Remainder of perilymphatic system, utricular macula and its innervation are omitted.

With a narrow tipped glass pipette, cold fixative was flushed through the saccule, emerging through the crus. The fixative was either 1% OsO_4 in veronal acetate buffer or 3-6% glutaraldehyde in phosphate buffer. Often one labyrinth was fixed in OsO_4 and the other in glutaraldehyde. The otic capsules were excised, transferred to fixative and the labyrinths rapidly removed and opened further. The saccular otolith was removed and fixation allowed to proceed for 1 hour. The tissue was washed in cold buffer and, with glutaraldehyde fixation, postfixed in phosphate buffered OsO_4 , dehydration in increasing alcohols and propylene oxide and embedding in epon followed. Further dissection of the individual organs was carried out in buffer or in alcohol. Thick sections (3μ) were viewed unstained or stained with toluidine blue in phase contrast. Thin sections were cut on an LKB Ultratome II, at 600-1000 Å, stained with uranyl acetate and lead citrate, and examined in a Hitachi HS-7S electron microscope.

RESULTS

The basilar papilla is a tubular evagination of the posterior wall of the saccule (Figs 1 and 2a). The tube terminates in a thin contact membrane which separates the endolymphatic and perilymphatic systems. The sensory epithelium consists of several parallel rows of hair cells, each row arranged semicircumferentially around the perimeter of the tube (Fig 2b). The sensory crest (Figs 2a, 3, 4) rises abruptly 60 μm from the contact membrane, reaches a height of about 30 μm , and gradually diminishes over a distance of 100 μm . Approximately 5 or 6 rows of sensory cells are seen, separated by supporting cells whose darkly staining nuclei lie close to the basement membrane. An earlier study (Geisler et al., 1964) has placed the number of hair cells in the papilla at about 60, in substantial agreement with our estimates. Hair bundles project from some of the hair cells, over them is the tectorial membrane, here shrunken and partially detached.

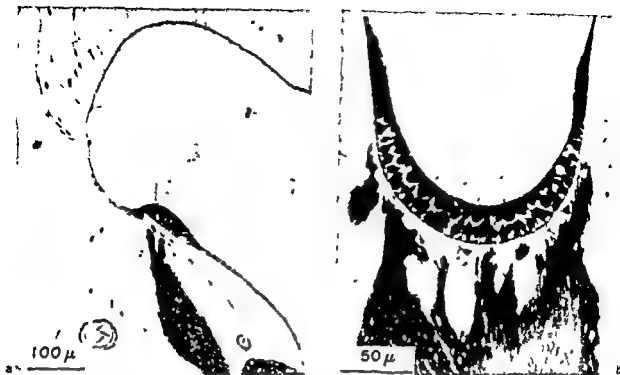


Fig 2 (a) Longitudinal section of basilar papilla. Contact membrane separates perilymphatic space on left from endolymphatic space on right. Tectorial membrane, overlying sensory crest, extends halfway across the lumen and bends near its top toward saccule. Myelinated nerve fibers approach papilla from below. Small blood vessels are seen in the cartilaginous wall. (b) Cross section of papilla through sensory epithelium. Sensory crest consists of hair cells and supporting cells, some of whose

nuclei are seen, several hair bundles are evident. Tectorial membrane extends across lower half of tube and is attached to walls of papilla.

In (a) and (b) open spaces occur in tectorial membrane, its detachment from sensory epithelium is probably due to shrinkage following fixation and dehydration. Nerve branch to papilla divides into smaller bundles and fibers lose their myelination just before entering sensory epithelium.



Fig 3 Longitudinal section of papilla at higher magnification (cf Fig 2a). Sensory crest, tectorial membrane, and part of contact membrane are seen. Nerve fibers approach sensory epithelium from below. Hair cell nuclei and hair bundles are evident near top of sensory crest. Nuclei below belong to supporting cells.

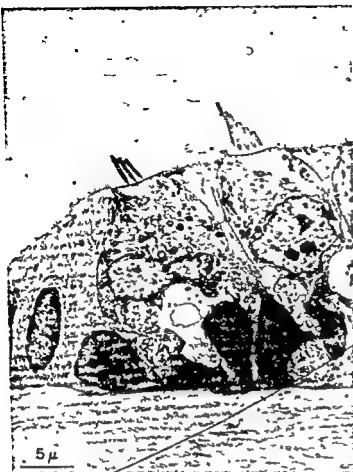


Fig 4 Two hair cells, corresponding to a portion of Figs 2a, 3. Stereocilia arise from cuticular regions of cells. Hair cells are separated by supporting cells from whose apical ends microvilli extend. Hair bundles were probably enclosed within spaces in tectorial membrane, now detached over most of its width. Cytoplasm of hair cells is distinguished by a variety of inclusions, not present in supporting cells. Nerve terminals containing mitochondria are seen between supporting cells abutting the basal ends of hair cells. Open spaces may be fixation artifacts.

by fixation. The hair bundles extend up into recesses in the tectorial membrane as suggested in Fig 3 by the bundle at left. The tectorial membrane appears to be firmly attached to the supporting cells just to the left of the first row of hair cells. At its upper free end the tectorial membrane is bent toward the saccule (Fig 2a) and is firmly attached at its margins to the wall of the papilla over most or all of this region of bending (Fig 2b).

The nerve bundle to the basilar papilla forms part of the posterior branch of the eighth nerve (Geisler et al., 1964). From the papilla the bundle courses toward the midline and then turns rostrally and after a short distance (200 μ m) joins the passing bundle from the posterior vertical canal. Branchlets from the amphibian papilla and lagena subsequently join the bundle

to form the posterior branch of the eighth nerve, which exits from the otic capsule through the posterior foramen. Within and close to the foramen is the posterior ganglion of the eighth nerve, containing the bipolar cell bodies of all the nerve branchlets including those of the basilar papilla. The nerve fibers thereafter take a medial course and enter the medulla, where they terminate.

In Fig 2b the myelinated nerve bundle approaches the sensory epithelium and divides into three or four smaller branches. The myelin sheath ends and the unmyelinated fibers pass through the basement membrane, to terminate on the hair cells (Figs 2-4). The fibers range in size from less than 1 μ m to about 5 μ m (Geisler et al., 1964) and number between 300 and 500. The nerve bundle flattens as it approaches

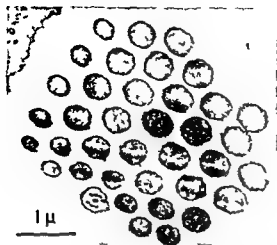


Fig 5 Cross section of hair bundle just above hair cell. Kinocilium is located on one side of bundle of stereocilia

sensory epithelium as can be seen by comparing Figs 2a and 2b. The plane of the ring of the sensory epithelium (Fig 2b) lies very close to a cross section through the animal. The tube of the basilar papilla is directed posterior and slightly lateral.

Hair cells are found in auditory vestibular and lateral line organs. A hair bundle consisting of a single kinocilium located to one side of a number of stereocilia protrudes from the apical end of each cell (Fig 5). The kinocilium is identified by a pattern—common to motile and sensory cilia—of nine double filaments surrounding a central pair of filaments. The stereocilia are arranged in a series of shallow rows behind the kinocilium. The axis of symmetry of this pattern passes through the kinocilium. The stereocilia decrease in height along this axis away from the kinocilium (Fig 4). Stereocilia in rows parallel to this axis show a similar decrease in height. Missing elements in the pattern may result from the fact that the plane of the section failed to intercept several of the smaller stereocilia. The row closest to the kinocilium contains five stereocilia instead of seven. In Fig 6 two stereocilia are partially seen in longitudinal section. They contain longitudinal filaments (Fig 5) which converge near the cell surface and extend into the cuticle of the hair cell, a non cytoplasmic plate found at the apical end

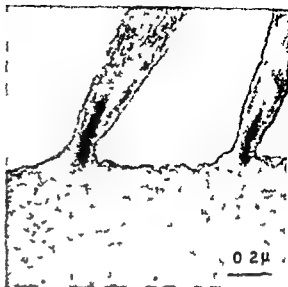


Fig 6 Longitudinal section of stereocilium entering cuticle. Cilia narrow as they approach cell and microfilaments converge to form a dense bundle which penetrates the cuticle for a short distance.

of the cell (Fig 4). The cuticle is notched and the kinocilium arises from the cytoplasm within the notch (Fig 7).

Our data show that the kinocilium is regularly located on the side of the hair bundle closest to



Fig 7 Longitudinal section through apical portion of hair cell showing kinocilium and several stereocilia. Within kinocilium several microtubules are evident, extending into hair cell. Kinocilium enters cytoplasmic portion of cell containing a variety of vesicles and other inclusions. To the left is the cuticle.



Fig 8 Afferent synapse of nerve terminal (right center) on hair cell (left). Dendrite contains many mitochondria. Within hair cell are a variety of membrane-bounded and electron-dense structures: mitochondria, vesicles, myelin figures and dense inclusions. At upper left is a portion of hair cell nucleus. Synaptic region of hair cell is characterized by dense synaptic body surrounded by clear vesicles of uniform size. Both pre- and postsynaptic membranes are thickened.

the contact membrane and most distant from the saccular opening of the papilla. This is suggested by the fact that in Figs 3 and 4 the hair bundles are longest on the side facing the contact membrane.

Afferent synapses are found on the hair cells (Fig 8). The synaptic region of the hair cell contains a dense, spherical synaptic body, approximately $0.25 \mu\text{m}$ in diameter, surrounded by a large number of synaptic vesicles (diameter about 400 \AA). The presynaptic membrane is thickened, troughs appear in the membrane under the synaptic body and contain dense rod-like structures (Fig 9). The synaptic body is flattened on the side facing the membrane. Post-

synaptically the membrane is also thickened and the afferent nerve terminals contain numerous vesicles and mitochondria.

Each hair cell synapses with several terminals (Fig 10) and one dendrite may branch and innervate several hair cells (Fig 11). We have estimated that there are approximately 60 hair cells innervated by 300 to 500 nerve fibers, so that each hair cell must synapse on average with more than five neurons. This result may be compared to the recent finding of Spoendlin (1971) that in the mammalian cochlea each inner hair cell is exclusively innervated by about 20 nerve fibers.

Efferent synapses described for other organs

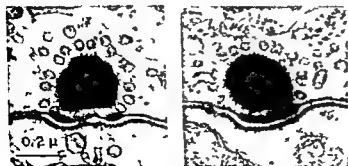


Fig 9 Sections through afferent hair cell-dendrite synapse. Hair cell contains synaptic body and vesicles above dense rod-like structures which lie in troughs in presynaptic membrane. Sections are from different synapses but are probably orthogonal cuts through similar structures.

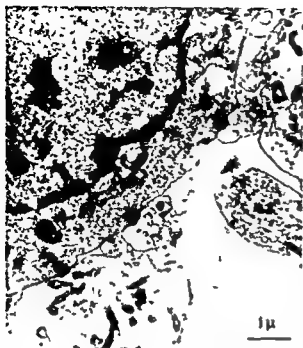


Fig 10 Hair cell with several afferent synapses each identified by synaptic body vesicles and thickened membranes

of the acustico lateralis system (Wersäll et al., 1965) have not been observed in the basilar papilla (Frishkopf & Flock 1967). Such synapses are characterized by a large number of densely packed vesicles within the nerve terminal close to the presynaptic membrane and by a closely apposed membranous sac within the hair cell

Although we searched for such structures we found only the type of synapse described above, corresponding closely to afferent hair cell-dendrite synapses found in other auditory and vestibular organs (Wersäll et al., 1965).

DISCUSSION

The input to the auditory papillae takes place by way of the tympanum, middle ear bones, and perilymphatic system. The latter is a membrane limited fluid filled space which envelops the endolymphatic system, in the region of the basilar and amphibian papillae the two systems are separated only by thin contact membranes (Figs 1 and 2a). Movement of the perilymph displaces the contact membrane and endolymph. The resulting tectorial membrane displacement acts through the sensory hair bundles to stimulate hair cells and nerve fibers.

A relation is known to exist in hair cells of the vertebrate acustico-lateralis system between the orientation of the sensory hair bundle as determined by the asymmetric location of the kinocilium and the direction of motion of the overlying structure (cupula or tectorial membrane) that is effective in stimulating the organ (for review see Flock, 1971). It has been shown for example that in the semicircular canals



Fig 11 Afferent nerve terminal synaptically contacting two hair cells. That these are in fact two hair cells was verified by examining a larger region of the section.

utriculo petal motion of the cupula increases the activity of nerves from the horizontal canal and decreases the activity from the vertical canals, utriculo fugal motion has the opposite effects. Examination of the orientation of the hair bundles with the electron microscope reveals that in the horizontal canal the kinocilium is located on the side of the hair bundle closest to the utricle while in the vertical canals it is located on the side of the bundle farthest from the utricle (Löwenstein & Wersäll, 1959). Thus it appears that displacement of the cupula in the direction from stereocilia to kinocilium increases—and opposite displacement decreases—neural firing rates.

A relation between orientation and functional properties has also been found in the lateral line canal organs of fish (Flock & Wersäll, 1962), where the gross electrical response from the organ to a sinusoidal stimulus contains a predominant second harmonic component. Electron microscopy reveals that neighboring hair bundles within the same organ are oppositely oriented. It is conjectured that oppositely oriented hair cells produce electrical responses that are 180° out of phase, summed over many cells the fundamental component cancels and only higher harmonics remain. A correlation between hair cell orientation, microphonic response, and nerve activity has also been found in the saccule of goldfish (Furukawa & Ishii, 1967*a, b*).

In the basilar papilla we have found that in each hair cell the kinocilium is located on the side of the hair bundle facing the contact membrane. Therefore, displacement of the tectorial membrane along the axis of the papilla should stimulate the organ, thus in the direction in which the tectorial membrane receives the maximum input from displacement of the contact membrane as well as the direction in which it presents maximum area (Fig. 2). We also expect displacement of the tectorial membrane toward the contact membrane to increase—and opposite displacement to decrease—the activity in afferent nerve fibers from the papilla.

On the basis of our observations, there appear to be no efferent terminals synapsing on hair

cells of the basilar papilla. Independent corroboration of this result has been obtained by degeneration studies (Robbins *et al.*, 1967), from these it appears that of the labyrinth organs in the frog only the basilar papilla lacks efferent innervation. Moreover, of all the organs of the acustico lateralis system so far examined, the basilar papilla is the only one that is so deficient.

It is interesting to note that whereas responses of basilar papilla afferents to acoustic stimuli are exclusively excitatory, the responses of amphibian papilla afferents to one tone can be inhibited by a second tone of higher frequency (Frishkopf & Goldstein, 1963). The possible role of efferents in this inhibitory process was previously discounted, based on the persistence of the effect after sectioning the eighth nerve. It is nevertheless intriguing to observe that of the two auditory organs found in amphibians only the one with efferent innervation exhibits inhibitory interactions. The possibility remains that efferent terminals may be involved in a local inhibitory process. This hypothesis may be tested by observing if inhibition is present at a time after cutting the nerve when efferent terminals have degenerated.

It is also noteworthy that the afferent nerve fibers from both basilar and amphibian papillae exhibit threshold vs frequency functions that rise steeply at about 100 dB/octave (Frishkopf & Goldstein, 1963). The origin of such steep tuning curves is uncertain, we can now be sure, however, that in the case of the basilar papilla efferents are not involved.

We have pointed out that the number of afferent nerve fibers to the basilar papilla greatly exceeds the number of hair cells; therefore the terminals synapsing on each hair cell must in general derive from several fibers. The innervation pattern is in that respect somewhat similar to that of inner hair cells in the organ of Corti (Spoendlin, 1971). The significance and function of this highly divergent innervation pattern is not known either in the basilar papilla or in the cochlea, but it could serve to increase the dynamic range of the organ.

ZUSAMMENFASSUNG

Die Papilla basilaris ist eines zweier Gehörorgane, die im Innenohr des Frosches gefunden werden. Licht und elektronenmikroskopische Untersuchungen der Papilla im Ochsenfrosch, *Rana catesbeiana*, haben frühere Beschreibungen ihrer grundsätzlichen Morphologie bestätigt und ausserdem neue Einzelheiten ihrer Feinstruktur aufgedeckt.

Die Papilla ist ein zylindrischer Auswuchs des Sacculus und erstreckt sich bis zu einer dünnen Kontaktmembran, welche die endolymphatischen von den perilymphatischen Räumen trennt. Das sensorische Epithelium überdeckt kammerförmig ein Halbbrund des Zylinders, und es wird überhangen von einer Tectorialmembran. Ungefähr 300 bis 500 myelinisierte Nervenfasern haben synaptischen Kontakt mit ungefähr 60 Haarzellen. Sensorische Haarbuschel entspringen von den Haarzellen und werden von Kanälen in der Tectorialmembran aufgenommen. Jedes Haarbündel besteht aus einer Gruppe von Stereocilien und einem einzigen Kinocilium, welches asymmetrisch der Haarzelle aufsitzt und zwar auf jener Seite des Haarbündels, die der Kontaktmembran zugewandt ist. An der Basis hat jede Haarzelle synaptischen Kontakt mit mehreren afferenten Nervenendigungen; efferente Synapsen konnten nicht gefunden werden.

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AN ELECTRON MICROSCOPIC STUDY OF ADRENERGIC INNERVATION IN THE COCHLEA

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Abstract By using electron microscopic techniques including the administration of a "false transmitter" it has been possible to identify adrenergic terminals filled with characteristic dense-cored vesicles in the cochlea. A continuous sympathetic perivascular plexus is found up to the limit of the habenula region. Adrenergic terminals are also found independent of blood vessels and this innervation is especially rich in the habenula region. Terminals are frequently found just before the demyelination zone and they surround the afferent nerve bundles when they have lost their myelin sheaths. In the habenula terminals are seen to make direct contact with non-myelinated afferent nerve fibres. Clusters of terminals are found in the tissue near the inner sulcus and toward the scala tympani. No adrenergic nerve terminals were found in the organ of Corti or vascular stria. The morphological localization of the sympathetic terminals support an influence on sound perception either via a direct action on sensory neurons or by vasomotor control.

The possible influence of the sympathetic nervous system on the cochlear and vestibular parts of the inner ear has been the subject of much interest from clinicians as well as anatomists and physiologists. An imbalance of the autonomic nervous system, in particular an overaction of the sympathetic part, has been discussed as a possible cause of diseases like Meniere's disease, tinnitus and perceptual deafness. Tympanosympathectomy (Lempert 1946, Rosen, 1951) and cervical sympathectomy (Passe, 1953, Lewis, 1964) have been performed in these conditions, occasionally with good results. However, very little

is known about the sympathetic innervation of the inner ear in man and the knowledge of the sympathetic innervation and its function in animals is limited.

The distribution of adrenergic nerve fibres in the cochlea has been difficult to investigate because of the special anatomy of the inner ear. It is enclosed in the temporal bone, and bony lamellae are closely adjacent to the innervated structures. Species differences, as well as considerable individual variation within species, have been claimed (Seymour & Tappin, 1951).

Using silver impregnation methods, a perivascular plexus plus the "plexus marginalis", consisting of non-myelinated fibres have been found in the cochlea (Lorente de No, 1926, Livan & Del Bo, 1951, Palumbi, 1954, Andrzejewski, 1955, 1956). The origin of these fibres was discussed and they were suggested to be of cerebral character or originating from sympathetic cervical ganglia. The results of these investigations were difficult to evaluate because it was almost impossible to distinguish between sympathetic nerve fibres and other thin non-myelinated nerve fibres. Mapping of the sympathetic innervation was made possible with the histochemical fluorescence method for catecholamines according to Falck and Hillarp (Falck et al., 1962). Using this method, Spoendlin & Lichtensteiger (1966, 1967) traced these fibres from the cervical ganglion to the inner ear via the tympanic

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plexus or the auricular branch of the vagus nerve. A dense plexus in the habenula region is of special interest since nerve action potentials are thought to be initiated in the habenula at the point where myelination starts. However, with light microscopy it is not possible to determine the exact location of the adrenergic terminals. In the electron microscope Spoendlin (1966) described what he supposed to be adrenergic terminals in this region. Electron microscopic studies by Terayama et al. (1968) did not support the presence of an adrenergic innervation in the habenula region and they concluded that the sympathetic innervation of the cochlea is confined to the vascular bed. Adrenergic fibres found between the myelinated nerve fibres, independent of blood vessels, were considered to run from one blood vessel to another. Special staining techniques are now available which make it possible to specifically identify biogenic amines at the ultrastructural level (Richardson, 1966; Tranzer & Thoenen, 1967). It is thus now possible to reliably identify fibres containing biogenic amines, and distinguish them from other nerve terminals. The loading of adrenergic fibres with a false transmitter, such as 5-hydroxydopamine (5-OH-DA), enhances the reliability of this method. It was the purpose of this investigation to localize the monoamine neurons in the cochlea and to describe their ultrastructural relationship to afferent nerve fibres and blood vessels, especially in the habenula region.

MATERIAL AND METHOD

Ten healthy cats weighing from 1.0–3.7 kg were used for the experiments. They were given either 5-OH-DA (3,4,5-trihydroxyphenyl ethylamine) 4×20 mg/kg or 5-OH-Dopa 3×200 mg/kg over a period of 48 hours (Tranzer & Thoenen, 1967). In a control animal unilateral cervical sympathectomy was performed about 3 weeks before it was sacrificed. The animals were anaesthetized with

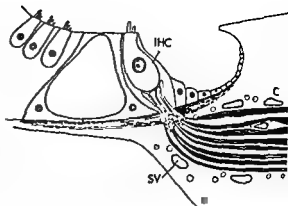
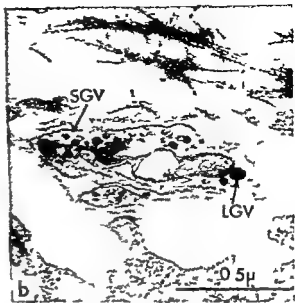


Fig. 1. Schematic diagram illustrating the adrenergic innervation of the cat cochlea. IHC, inner hair cell; SV, spiral vessel of the tympanic lip; C, capillary; O, adrenergic nerve fibre.

2.5% Nembutal before decapitation. The cochleas were rapidly dissected out and opened widely to obtain good contact with the fixation medium. Fixation was performed in ice-cold 3% potassium permanganate (0.1 M sodium phosphate buffer, pH 7.0) (Richardson, 1966; Hokfelt & Jonsson, 1968; Hokfelt, 1969). After rinsing in Ringer solution the pieces were contrasted en bloc in 1% uranyl acetate-Ringer solution. In several cases the cochlea on one side was fixed in 3% glutaraldehyde (0.133 M sodium phosphate buffer, pH 7.3) followed by Veronal acetate buffered 1% osmium tetroxide solution (Rhodin, 1954) and in a few cases fixation was obtained by vascular perfusion, first with Ringer solution, then with glutaraldehyde. The pieces were dehydrated in ethanol and embedded in Epon (Luft, 1961). For electron microscopy, sections were cut with glass knives on an LKB Ultratome. Sections from specimens fixed in potassium permanganate were stained with lead citrate (Watson, 1958) and those fixed in osmium tetroxide were stained with uranyl acetate (Reynolds, 1963) followed by lead citrate. Sections were taken from the modiolus, spiral ganglion, spiral osseous lamina, habenula, organ of Corti, and from the stria vascularis and were examined in a Siemens Elmiskop I electron microscope.



Fig 2 Adrenergic nerve terminals have different appearance depending on fixation procedure. Pre-treatment with 5-OH DA. (a) Fixation in potassium permanganate. The vesicles have a distinct dense core



× 51 000 (b) After fixation in glutaraldehyde and osmium tetroxide the vesicles are completely filled with an osmiophilic substance × 42 500 LGV: large granular vesicle SGV: small granular vesicle

RESULT

In the cochlea two systems of adrenergic innervation were found one along blood vessels and one consisting of terminals freely distributed in the modiolus, spiral ganglion, spiral lamina and in the habenula. The distribution pattern is illustrated in Fig 1.

Appearance of terminals

As in other areas the adrenergic nerve fibres of the ear are characterized by the presence of local swellings of typical appearance so-called varicosities which are found along the thin non myelinated axon in increasing numbers towards its periphery. Each varicosity is named a terminal even though it does not usually terminate the axon. Generally only the terminals are seen in a single section, but in serial sections the interconnecting nerve fibre is observed. Each terminal contains an abundance of dense-cored vesicles the centre of which stains characteristically after fix-

ation with potassium permanganate (Fig 2a) (Richardson, 1966; Hokfelt, 1969). The diameter of the vesicles is about 500 Å but a few with a diameter of 1000 Å are also encountered. Most of the vesicles appear empty if the animal is not pretreated with the false transmitter 5 OH DA (or its precursor 5 OH Dopa). The number of vesicles differs considerably between terminals, so while some are densely packed others may contain only a few. This difference is independent of pretreatment with 5 OH DA or 5 OH Dopa. After fixation with glutaraldehyde and osmium tetroxide the vesicles appear almost filled with a dense osmiophilic material (Fig 2b) (Tränzer & Thoenen, 1967).

Perivascular innervation

An adrenergic perivascular plexus was found in sections from the modiolus, spiral ganglion, spiral lamina and around the spiral vessel of the tympanic lip (Figs 3 and 4). Terminals close to blood vessels were initially



Fig 3 Blood vessel in the spiral ganglion with adrenergic innervation. Fixed in osmium tetroxide. CL

capillary lumen EC endothelial cell NT adrenergic nerve terminal $\times 46\,500$

not completely covered by a Schwann cell and were situated at varying distances from the vessels. The distances were sometimes as small as 1 000 Å or less but very often the terminals were situated at distances of 3 000 Å or more. Generally there were several nerve terminals situated at different distances from the vessel. In potassium permanganate fixed material a second type of nerve ending containing non granulated small vesicles of smaller diameter was sometimes observed in the vicinity of adrenergic terminals close to blood vessels.

In the *stria vascularis* no adrenergic terminals or cells containing catecholamines could be found either around blood vessels or elsewhere in the tissue.

Blood vessel independent innervation

Spiral ganglion In the spiral ganglion adrenergic terminals were found independent of blood vessels. Such terminals were seen in close proximity to myelinated ganglion cells (Fig 5). There was no special sympathetic innervation of type II ganglion cells of the non myelinated type (Spoendlin 1971). Axons and terminals



Fig 4 Blood vessels (BV) in the spiral lamina also have adrenergic innervation. Fixed in potassium permanganate. CL capillary lumen. EN endothelial nucleus. NT adrenergic nerve terminal. $\times 43\,000$.



Fig 5 An adrenergic nerve terminal (NT) between two myelinated ganglion cells (GC) in the spiral ganglion. Potassium permanganate, $\times 27\,000$.



Fig. 6a. Section from the cochlea with the demyelination zone and inner hair cell (IHC). Fixed

in osmium tetroxide. St. spiral vessel of the tympanic lip. $\times 3400$.

were ensheathed by Schwann cells which left part of the membrane of the terminals free.

Lamina spiralis. On their course towards the organ of Corti afferent nerve fibres lost their myelin sheath in the region of the habenula perforata where bundles of fibres left the lamina spiralis ossea. Just before the demyelination zone free terminals were seen above and below the myelinated nerve fascicles and also interspersed between the fibres. Adrenergic terminals and axons usually appeared without a Schwann cell envelope, but might sometimes be totally or partly surrounded by Schwann cells.

In the habenula where demyelination occurred, bundles of axons were surrounded by

adrenergic terminals. Terminals were constantly found below (Figs 6 and 7) and between the nerve fibres (Fig. 8). In between the unmyelinated part of the axons slender terminals were sometimes observed, containing the typical dense-cored vesicles. Some terminals were located in direct contact with the non myelinated part of the axons (Fig. 9). Morphological evidence of a direct synaptic contact with pre- and post synaptic membrane specialization, between adrenergic terminals and afferent dendrites was not obtained. Terminals in this region were generally completely free of a Schwann cell envelope. Free terminals, or even clusters of terminals were also present at a greater distance from sensory fibre



Fig 6b Higher magnification of the framed area in Fig 6a showing the relationship between a nerve terminal (NT) and demyelinating nerve fibre (NF) $\times 25\,300$

bundles namely above the fibres in the connective tissue below the tympanic lip of the limbus and below the fibres in the connective tissue towards the scala tympani (Fig 1) Terminals were also seen between the extension of fibroblasts below the basement membrane at the level of the inner hair cell (Fig 10)

Organ of Corti Adrenergic terminals were not found in the inner spiral plexus below the inner hair cells, in the tunnel of Corti, or at the level of the outer hair cells

The unilaterally sympathectomized cat got a paralysed nictitans membrane on the operated side No adrenergic terminals were found anywhere in the cochlea on that side On the non-operated side there was a normal distribution of adrenergic terminals

DISCUSSION

Since the introduction of the fluorescence method by Falck and Hillarp (Falck et al,

1962) the cochlear sympathetic innervation has been investigated by fluorescence microscopy Having found fluorescent sympathetic nerves both close to, and apart from blood vessels, the question has been to what extent the blood vessels in the cochlea are innervated and whether there is a system of terminals independent of blood vessels Spoendlin & Lichtensteiger (1967) showed a perivascular plexus out to the periphery of the modiolus Terayama et al (1965, 1966) found fluorescence around the spiral vessel too Electron microscopic studies using standard osmium fixation have also been performed, where unmyelinated nerve fibres have been mistaken for adrenergic nerve fibres (Ross, 1971) Terayama et al (1968) described terminals around blood vessels in the radiating cochlear bundles However, they found none around the spiral vessels or in the habenula region

It has been shown (Hökfelt & Jonsson, 1968) that a reaction occurs between certain



Fig. 7. Adrenergic nerve terminals (NT) in the region surrounding nerve fibres (NF) entering the organ of

Corti. Fixed in potassium permanganate. BM, basilar membrane below inner hair cell. $\times 11800$.

fixatives such as KMnO_4 and several biogenic amines resulting in a precipitate depending upon the concentration of the amine. Using routine electron microscopic techniques with glutaraldehyde and/or osmium tetroxide as a rule only a few dense-cored vesicles are found in several tissues. Incubating the tissue

in an amine containing medium or administration of a MAO inhibitor or a 'false' transmitter such as 5-OH-DA gives a concentration high enough to produce a distinct precipitate within the vesicles (Hökfelt, 1968, Tranzer & Thoenen, 1967). Potassium permanganate has the advantage of demonstrating adrenergic ve



Fig 8 Adrenergic nerve terminals (NT) are seen where myelinated nerve fibres (NF) lose their myelin sheaths Potassium permanganate $\times 31\,800$

sicles unmistakably but also has the disadvantages of difficult sectioning and poor tissue penetration. It is difficult to get a high enough concentration in the modiolus and spiral ganglion to react with the transmitter. In the cochlea, however, where the tissue is thin, the reaction between the fixation medium and transmitter is very good. Potassium permanganate is a vigorous fixative which destroys certain tissue components, giving an inferior morphology compared with that after fixation with glutaraldehyde followed by osmium tetroxide. Glutaraldehyde and osmium tetroxide give superior preservation of deep structures due to better penetration. In the present study, a false transmitter has been used.

A continuous perivascular plexus is found in the modiolus along the vessels in the radiating cochlear nerve bundles and around the

spiral vessel in the tympanic lip. It is difficult to determine how far from a vessel a terminal can be situated and still exert an effect. In investigations on the sympathetic innervation of the intestinal and mesenteric vessels, terminals are found at varying distances from the vessels. It has been concluded that an effective influence can be exerted at a distance of about $1\ \mu$ (Devine & Simpson 1967). In the cochlea, the terminals are also found at varying distances from the vessels. No direct contact can be shown between nerve terminals and vessels, but the terminals are sometimes found at a distance of only $0.1\ \mu$ from the blood vessel. One way in which the sympathetic nervous system may influence sound perception is via an action on the cochlear vascular supply.



Fig. 9. Adrenergic nerve terminal (NT) in direct contact with a demyelinated nerve fibre (NF). Free

nerve terminals are seen (NT₂). Potassium permanganate $\times 35000$.

Apart from the vascular sympathetic innervation an adrenergic innervation independent of blood vessels exists. Using the stretch preparation technique Spöndlin & Lichten

steiger (1967) found a terminal plexus in the habenula region. The density of the fibres was highest at the basal part of the cochlea. With the fluorescence technique Terayama

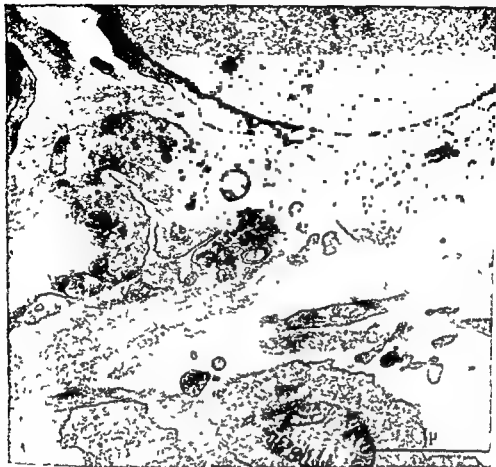


Fig 10 Adrenergic nerve terminals among fibroblasts towards the scala tympani at the level of the inner hair cell. Potassium permanganate $\times 46\,800$

et al (1965, 1966) found fibres in the habenula region which they interpreted as running from one blood vessel to another. With electron microscopy they did not find any adrenergic terminals in this region. In the present study adrenergic terminals are found in the spiral limbus between the myelinated nerve fibres and are richly distributed between the nerve fibres just before the demyelination zone. When the demyelinated afferent nerve fibres penetrate the habenula the bundles of axons are surrounded by adrenergic nerve terminals containing dense-cored vesicles. Terminals are also situated between the non myelinated dendrites, sometimes in direct contact with

nerve fibres. Today no method exists, however, which makes it possible to show post synaptic membrane specialization. Distal to the habenula no sympathetic innervation is found in the organ of Corti or in the stria vascularis. In the electron microscope, nerve terminals along non myelinated afferent nerve fibres and the inner spiral bundle contain a large number of empty vesicles and few with a dense core. This is a constant finding in osmium tetroxide fixed specimens, whether the animal is pretreated with a 'false transmitter' or not, and after sympathectomy. In KMnO_4 fixed specimens these dense cored vesicles appear empty or only weakly stained.

They are considered to be large granulated vesicles not containing biogenic amines.

Apart from this direct innervation of blood vessels and afferent nerve fibres there is a diffuse distribution of adrenergic terminals in the tissue towards the scala tympani and towards the inner sulcus. Spoendlin & Lichtensteiner (1967) suggested a sympathetic influence in the habenula region on the threshold for impulse generation in afferent nerve fibres. Much work has been done to find out in which way the sympathetic nervous system influences different sense organs. In amphibians Hutter & Lowenstein (1955) and Lowenstein (1955) found a reduced threshold for impulse generation in terminals of touch receptors and a facilitated neuromuscular transmission when the sympathetic chain was stimulated.

By stimulating the cervical sympathetic ganglion, Beickert et al. (1956) showed a decrease in the cochlear microphonic potential. Seymour & Tappin (1951) found an initial increase followed by a decrease of microphonics during sympathetic stimulation. However, others (Rambo et al., 1953) did not find any effect on cochlear microphonics, either after stimulation or after sympathectomy. Russell (1969) when recording the spontaneous afferent nerve impulses from the lateral line organs in *Xenopus* could not discern any change in discharge frequency during stimulation of the sympathetic trunk or after local administration of adrenalin-noradrenalin.

Since no adrenergic terminals are found close to hair cells in the organ of Corti the sympathetic influence on cochlear microphonic potentials must be very small or even negligible, which might explain the contradictory results cited above.

The presence of many adrenergic terminals in the habenula region and the short distance between them and the afferent nerves at the demyelination zone suggest the possibility of a sympathetic influence on sound perception.

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ZUSAMMENFASSUNG

Unter Verwendung der elektronenmikroskopischen Technik und Verabreichung einer falschen Transtorsubstanz konnten in der Cochlea adrenerge Nervenfortsätze mit charakteristischen dichten kernhaltigen Bläschen entdeckt werden. Ein kontinuierlicher sympathischer perivaskulärer Plexus wird bis an die Grenze der Habenularegion hin vorgefunden. Adrenerge Nervenfortsätze kommen auch unabhängig von Blutgefäßen vor, und diese Innervation ist besonders reichlich in der Habenularegion. Nervenfortsätze finden sich häufig unmittelbar vor der Zone der Entmyelinisierung und umgeben die afferenten Nervenbündel nach Verlust ihrer Myelinscheide. In der Habenula sind Nervenfortsätze zu finden, welche direkten Kontakt mit den myelinfreien afferenten Nervenfasern haben. Anhäufungen von Nervenfortsätzen zeigen sich im Gewebe in der Nähe des inneren Sulcus und der Scala tympani. Keine adrenergen Nervenfortsätze wurden hingegen im Cortischen Organ und in der Stria vascularis gefunden. Die morphologische Lokalisation der sympathischen Nervenfortsätze erhartet ihren Einfluss auf die Schallperzeption entweder durch direkte Wirkung auf die sensorischen Neuronen oder auf dem Wege einer vasomotorischen Steuerung.

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APPLICATION OF IMPEDANCE AUDIOMETRY AS A SCREENING INSTRUMENT

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Abstract More than seven hundred Southeast Alaska pre school and grade school children were screened with pure tone audiometry and abbreviated acoustic impedance measurements. Impedance data was obtained on 94.8% of the children while audiometric data on only 70.3% due to early age or lack of cooperation. Over 40% of the children failed impedance screening criteria. Of these 70% had Type C tympanograms and 30% had Type B. Abnormal impedance results were found in nearly 10% of the children who passed audiometric screening. Because impedance testing is particularly suitable to the high risk pre school and early grade school population and because it tests for disease and not just hearing loss, the authors recommend its addition to pure tone testing in screening programs. We would go so far as to say that in younger children if only one were available impedance testing would be preferred.

The primary purpose of a hearing conservation program for children is to identify as many correctable ear conditions as possible in order to prevent the development of hearing loss which might interfere with social and educational growth and development. Conventional pure tone audiometric screening has been assumed to be able to reveal not only children with hearing loss but also those with ear disease which should be referred to medical care. Data from the classical Pittsburgh study of Jordan & Eagles (1961) indicated that audiometric screening however complete, can not identify all children with ear disease who

need medical treatment. In that study over half of 629 otologically abnormal ears had hearing sensitivities equal to or better than zero dB ASA.

Jordan & Eagles (1961) have also shown that many children normally hear at levels below audiometric zero. Thus a child may experience a significant decrease in hearing sensitivity without this decrease being detected by the usual audiometric screening.

In our early experience with pure tone audiometric screening in the schools of Southeast Alaska we noticed that many individuals who had "passed" the screening at a level 25 dB re ANSI were identified by an examining physician or otologist to have a middle ear disease which required medical or surgical treatment. It became evident that new screening techniques were needed to properly identify these children.

It was the purpose of this study to evaluate the use of acoustic impedance measurements in conjunction with pure tone screening as a means of identifying children with middle ear disease needing treatment. While acoustic impedance measurements have been made for years, their utilization as a screening device to detect middle ear disease has been a relatively late development. Brooks (1969) tested over 1 000 five to ten year old English school children by this means and found it a simple

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and effective means of detecting middle ear fluid. According to his data, the incidence of middle ear fluid decreased as age increased.

SUBJECTS

The study population consisted of 710 children enrolled in 10 pre schools and four elementary schools in Southeast Alaska. They were grouped for the study into three groups: pre schoolers, grades kindergarten through three, grades four through twelve. Pre school children were five years old or younger, children in kindergarten through third grade ranged from ages five through nine, and children in fourth through twelfth grade were 9 years of age or older.

METHODS

To insure an unselected population, all children in each of the 14 schools were screened utilizing both pure tone and acoustic impedance measurements. Pure tone screening was accomplished by using a Maico 2A portable audiometer, and acoustic impedance measurements were accomplished by using either the Madsen ZO 70 or ZO-72 electro-acoustic impedance meters. Both the Maico audiometer and the acoustic impedance meters were calibrated using a Bruel & Kjer sound level meter prior to each school screening.

The pure tone screening consisted of checking for patient's response to pure tones of 500, 1 000, 2 000 and 4 000 Hz at a level of 25 dB re ANSI. This level of presentation was chosen because the tests all had to be administered in an empty classroom without sound treatment and ambient noise was higher than desirable.

Acoustic impedance measurements consisted of the following:

- 1 Inserting the probe tip and obtaining an air tight seal.
- 2 Introducing a positive pressure of 200 mm H₂O into the ear canal and then checking the seal by monitoring the manometer.

- 3 Taking the reading in acoustic ohms at this pressure reading of -200 mm.
- 4 Reducing the pressure until the peak compliance of the tympanic membrane was noted by the swing of the balance meter from the left to the right.
- 5 Noting the tympanogram type by ascertaining the middle-ear pressure reading at that point.
- 6 Taking the reading in acoustic ohms at this level of peak compliance.
- 7 At the most compliant position presenting to the contralateral ear a 1-2 second 100 dB HTL pure tone stimulus of 1 000 Hz, and noting the presence or absence of the acoustic reflex as indicated by the deflection of the balance meter.
- 8 Calculating the static acoustic impedance from the two previous acoustic ohms readings by the use of a Madsen nomogram.
- 9 Repeating the process on the other ear.

The criteria for failure of pure tone screening consisted of failure to respond to tones presented at 25 dB ANSI at frequencies of 500, 1 000, 2 000 or 4 000 Hz in either or both ears.

Three criteria for failure of impedance screening were chosen on the basis of previous experience:

- 1 A Type A tympanogram with an absent acoustic reflex.
- 2 A Type B tympanogram.
- 3 A Type C tympanogram.

The static impedance values were not used as a criterion for failure of acoustic impedance screening because of the frequent overlap of normal and abnormal values in many disease states.

Jerges's classification of tympanograms into Types A, B and C was used for simplicity of description. A Type A tympanogram exhibits maximum tympanic membrane compliance when the pressure in the external auditory canal is near zero. The pressure at this most compliant point may be slightly negative but

does not equal or exceed -100 mm H₂O. A Type B tympanogram exhibits little or no compliance change over wide pressure variations in the external ear canal. A Type C tympanogram has a compliance maximum in the negative pressure range between -100 and -400 mm H₂O.

RESULTS

Of the 710 children in the study, audiometric pure tone screening results could not be obtained on 29.7% of the children because of the early age of the child (35 were under the age of two) or because of the child's lack of cooperation (Table I).

It was possible to obtain acoustic impedance results on all but 1.3% of the children providing results on 1401 ears (Table II). Of these 41.5% failed our criteria for acoustic impedance screening. Of the total 13.0% had Type B tympanograms and 28.5% had Type C tympanograms. There were no instances of Type A tympanograms with absence of the acoustic reflex although we have seen this in older children, adults, and some young children not included in this study. (By our testing method the acoustic reflex was always present in ears with Type B tympanograms but remained elicitable in one third of those ears with Type C tympanograms.) The highest percentage of abnormalities was seen in pre-school and young school age children with the lowest percentage found for the fourth through twelfth grade group. In the kindergarten through third grade group just over 50% had either Type B or Type C tympanograms.

Of those who could be tested by pure tone screening 97% had "normal hearing" but

Table I Number of patients unable to be audiometrically screened

Pre school	171
Kind 3rd	76
4th 12th	0
Total	147

Table II Results of impedance screening

	A	B	C
Pre school			
R ear	239	88	116
L ear	240	49	124
Kind 3rd grade			
R ear	77	23	57
L ear	77	23	56
4th 12th grade			
R ear	96	13	22
L ear	93	17	21
Total	822	183	396
% of total ears	58.5	13.0	28.5
Total* patients	710		
Total ears	1401		

* No results could be obtained on 9 patients or 1.3% of total seen.

failed the acoustic impedance screening (Table III). With one exception these were all in the pre-school and kindergarten through third grade groups. This 9.7% represents children who but for the acoustic impedance results would not be referred for appropriate medical attention.

DISCUSSION

The incidence and magnitude of middle ear disease in Alaska is staggering. The cohort study of Reed et al. (1967) showed that nearly two of every three Eskimo babies had experienced purulent otorrhea from acute suppurative otitis media by the age of 2 years. Harker & Van Wagoner (1972) in a 3 month period of observation found 15% of a mixed racial Southeast Alaska grade school population had either middle ear effusion or persistent abnormal negative intratympanic pressure indicative of eustachian tube malfunction. A mixed racial survey by Hayman &

Table III Number of children with normal hearing failing impedance screening

Pre school	56
Kind 3rd	10
4th 12th	1
Total	67
% of total screened	9.7%

Kester (1956) revealed 53% of 900 children had perforation, retraction, scarring, or inflammation of the tympanic membrane. Our high percentage of abnormal test of middle ear function thus did not surprise us.

Seventy percent of ears failing our impedance screening criteria had Type C tympanograms which reflect negative intratympanic air pressure. This occurs because the eustachian tube fails to open during swallowing. The air in the tympanic cavity, which is constantly being absorbed via mucosal capillaries, is no longer replenished, and a partial vacuum results. The causes of this eustachian tube malfunction are many; some are short-lived and recovery may occur without treatment, but others may never resolve without medical or surgical treatment. Ideally all these patients should be seen by an otologist.

Thirty percent of the ears failing the impedance screening exhibited Type B tympanograms with a flat tympanic membrane compliance function. If the tympanic membrane was intact and the child had no previous surgery on the test ear, middle ear fluid was nearly always present, although its viscosity was sometimes thick and sometimes thin. Otological referral and treatment is always indicated with a flat compliance curve. It was extremely rare in our experience for a Type B tympanogram to revert to a Type C, but the opposite was common and represented progression of disease.

Some pre school children can be rapidly conditioned to respond to pure tones and a quick valid screening can be obtained. More often it is time consuming and considerable experience and skill are required to obtain valid results. Sometimes it is just impossible. It was encouraging to get meaningful data on middle ear function by impedance testing in such a high percentage of these children. The pre school and early school grades had the highest incidence of impedance screening failures and it was almost exclusively in these younger groups where some children passed audiometric screening but exhibited abnormal

impedance measurements. Impedance screening represents an effective means of increasing the detection rate of middle ear disease needing otologic referral in these younger children. The goal of screening should be the detection of disease, not just the detection of those diseases which at the time of the test are accompanied by hearing loss.

ZUSAMMENFASSUNG

In der vorliegenden Studie wurden über 700 Vorschul- und Grundschulkinder in Südost-Alaska mit Tonaudiometrie und einem vereinfachten Impedanzmessverfahren im Screeningtest getestet. Impedanzdaten zeigten, dass 8% der Kinder aufgerommen werden während dagegen audiometrische Daten nur von 70,3% erhalten werden konnten, weil die Kinder zu jung waren oder nicht entsprechend mitarbeiteten. Bei über 40% der Kinder fielen die Impedanzdaten außerhalb des Rahmens der Impedanzkriterien im Screeningtest. Von dieser Gruppe hatten 70% Tympanogramme vom Typ C und 30% vom Typ B. Anormale Impedanzwerte wurden bei nahezu 10% der Kinder, bei denen audiometrisches Screening durchgeführt werden konnte festgestellt. Da sich der Impedanztest in Risikofällen bei Vorschulkindern und jüngeren Grundschuljahrgängen als gut anwendbar erwiesen hat und da er gewissermaßen die Basis der Schädigung und nicht den Hörverlust als solchen testet, schlagen die Autoren diesen als Zusatztest zu den tonaudiometrischen Tests im Screeningprogramm vor. Sollte bei jüngeren Kindern nur ein Test durchführbar sein, so ist nach Ansicht der Autoren dem Impedanztest der Vorzug zu geben.

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A COMPARATIVE STUDY OF THE DEVELOPMENT OF HEARING AND VISION IN VARIOUS SPECIES COMMONLY USED IN EXPERIMENTS

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Abstract Inception of hearing and opening of eyelids were examined in cat, rabbit, dog and mink. The hearing tests were performed with pure tones and bands of noise observing various types of behavioural responses. Hearing was found to occur at an average age of 5 days in cat, 7 days in rabbit, 14 days in dog and 29 days in mink. The frequencies first responded to and the successive development of hearing for other frequencies are reported and related to data from the literature for five other species. Opening of eyelids started at an average age of 7 days in cat, 10 days in rabbit, 14 days in dog and 33 days in mink. The possible connection between blood supply to the various coils of the cochlea (in man) and the ontogenetic development of hearing and the deterioration of hearing with aging and acoustic trauma is discussed.

The age at which hearing and vision occur in different species has been the subject of several publications. *hearing* (Wada, 1923, Larsell et al., 1935, 1944, Rose et al., 1957, Marty, 1962, Alford & Ruben, 1963, Anggård 1965, Mikaelian & Ruben 1965, Crowley & Hepp-Reymond 1966, Hilding et al., 1967, Pujol et al., 1967, Pujol & Marty, 1968, Flottorp & Foss, 1970, Foss & Flottorp, 1970, Sugiura & Hilding 1970, Johansson, 1972, Johansson et al. 1972) *vision* (Contino, 1907, Jasper et al., 1937, McCrady 1938, Hunt & Goldring, 1951, Marty 1962, Rose, 1968, Rose & Ellingson, 1968, Erichsen, 1969).

Rarely have the authors tried to correlate their observations about the start of functioning of one sensory modality with another,

or with other characteristics, such as weight of the animal. The age of an animal may not be the only or best parameter referred to in such studies. At least as much information is obtained when age is correlated with body weight.

Some investigators have been concerned with the frequency range to which the animal has first responded (Larsell et al., 1935, 1944, McCrady et al., 1937, 1940, Mikaelian & Ruben, 1965, Anggård, 1965, Crowley & Hepp-Reymond, 1966, Pujol et al., 1967). However, this problem has most often been neglected. The investigators have shown little interest for correlating their observations of the development of response in the sense organ with the blood supply to the sense organ.

The object of the present study was to determine when hearing and vision occur in various species and to correlate these data with the animals' weight. We have also tried to determine the frequency range first responded to. In addition we discuss our findings with respect to the representation of frequencies along the basilar membrane and to the blood supply of the inner ear.

MATERIAL AND METHODS

We have examined four different species: cat, rabbit, dog and mink, using animals from dif-

Table I Number of animals at various ages submitted to hearing test

Species	Litter no	Litter size	Animals tested			Age (in days) when submitted to hearing test																				No of hearing tests	
			No	Sex		2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	20	22	24			
				♂	♀																						
Cat	1	2	■	1	1	2	2	2	2	2	2															14	
	■	1	1	1	1							1	1	1	1	1										5	
	3	4	4	3	1		4	4	4	4	4	4	4	4												32	
	4	4	4	2	2	4	4	4	4	4	4	4	4	4	4	4										44	
Rabbit	1	7	2	1	1				2	2	2	2	2	2	2	2										16	
	2	7	2	1	1				2	2	2	2	2	2	2	2										16	
	3	6	2	1	1				2	2	2	2	2	2	2	2										16	
Dog	1	6	5	3	2													5	5	5	5	5	5	5	5	40	
Age (in days) when submitted to hearing test																											
26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43																											
Mink	1	4	2		2				1	1	1	1	1		1	1	1	1	1	1		1	1			13	
	2	2	2	1	1			1	1	1	1	1	1	1	1	1	1	1	1	1		1	1			13	
	3	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2					16	

ferent litters—in cat, four litters, in rabbit and mink, three litters, and only one litter in dog (see Tables I and II)

Our cats are of the usual mixed breeds—house cat (*Felis catus*)—with rather substantial variation in number of kittens in each of the four litters, and gestation length unknown, although the birth date is known. None of the kittens were white (suggestive of hereditary deafness).

The rabbits are well defined, being a New Zealand white (albino with red eyes), gestation length of all three litters being precisely 31 days, equal to reported average length (Erich-

sen, 1969), and litter size (6.7) being very close to reported average of 7 (Erichsen, 1969).

The mink is the dark mink (standard). Gestation length was 46.3 days on average. This was of little importance, however, since the development of the fetus varies substantially from mother to mother. The average gestation length in standard mink is 49.1 days (Udris, 1970). The average size of our litters was 2.7, somewhat smaller than reported by Udris (1970) 5.2.

The dog is the Norwegian Dunkerhound, the gestation length 64 days, close to the reported average 63 days (Erichsen, 1969). Litter size was 6, very near to the average size for Dunkerhound 6.9 (Lyngset & Lyngset, 1970). Only puppies of black and merle colour were used in the present work, excluding a white puppy (which forms part of a study of hereditary deaf animals to be published later).

The hearing tests have been performed in an approximately anechoic sound proof chamber, using a Peter's Audiometer, type AP 5 together with a Peter's Power Amplifier and Loudspeaker, type AS 11.

Table II Number of animals tested and number of hearing tests performed

Species	No of animals tested	No of hearing tests
Cat	11	55
Rabbit	5	48
Dog	5	40
Mink	6	42
Total	28	225

The animal was placed in front of the loud speaker either on a resilient pad or held in the hands of the experimenter (see Fig 1 for the test situation). The younger the animal, the more necessary it was found to let it feel the warmth of the hands of the experimenter, in order that it was sufficiently relaxed for the test.

Pure tones (125–8 000 Hz in octave steps, plus 750 Hz), and narrow band of noise centered at 750 Hz (approximately 1/3 octave) and "white" noise (limited by the loudspeaker's frequency response) were used as test stimuli at intensities shown in Table III.

A positive response of the animal to the signal was judged to be either a startle or varying intensity, a pinna or cochleo-orbicular reflex or some type of "behaviour change" evaluated according to strength, as presented in Table III. The test was rather time-consuming as it was necessary to observe the animal very carefully as to the kind of "status" it had before applying the stimulus. By experience we learned that for instance no sound production from the animal could be accepted during the testing period. The stimulus had to be repeated sufficiently often in order to ensure the validity of the response. Both authors served as observers, one of us controlling the apparatus (stimulus), the other one keeping the animal in correct position.

In order to perform a thorough hearing test, we found it necessary to limit the number of animals to about 6 on each test day. In another study we manage to test 12 animals each day. However, this was found to be rather tiring for both experimenters and animals. Of the 20 rabbits from 3 litters we picked out one of each sex at random from each litter. All tests were performed during the morning hours, observation of the eyelids and of the weight after finishing the hearing test.

Ophthalmoscopy was used in dog and mink in order to examine whether opening of the eyelids corresponded with the ability to see, i.e. presence of the orbicular reflex (contrac-



Fig 1 Animal (cat) under test in front of loud speaker

tion of *m orbicularis oculi*) and of pupil variation (contraction of *m sphincter pupillae*) as a response to light. In observing the status of the eyelids we employed a three category scale:

- 1 Beginning. When a fissure could just be observed, always starting at the medial angulus.
- 3 Full Opening completed both laterally and vertically.
- 2 Half. The stages in between 1 and 3.

The weight of the animals was measured in grams, using a Mettler Precision Balance model P 1200.

RESULTS

The results of the hearing tests of one animal are shown in Table III (cat), and for all

Table III *Result of hearing test of one animal (cat)*

	Age (days)										
Test stimulus	2	3	4	5	6	7	8	9	10	11	
White noise (103 dB SPL)	-	-	-	-	-	-	-	+-	++	+++	
Noise band 750 Hz (113 dB SPL)	-	-	-	-	-	(+)	++	++	++	+++	
Pure tone audiometry											
Frequency (Hz)	Sound level (dB SPL)										
125	120	-	-	-	-	-	(+)	++	-	++	
250	120	-	-	-	-	-	++	(+)	++	++	
500	118	-	-	-	-	-	-	++	++	++	
750	115	-	-	-	-	-	-	+	++	++	
1 000	108	-	-	-	-	-	-	-	++	++	
2 000	103	-	-	-	-	-	-	-	++	++	
4 000	106	-	-	-	-	-	-	(+)	+	++	
8 000	105	-	-	-	-	-	-	-	(-)	+	

+++ Extremely strong startle reflex

++ Easily recognized startle reflex or pronounced pinna reflex

+ Less pronounced startle, pinna, cochleo-orbicular reflex or 'behaviour change

(+) Uncertain response

- No response

animals in Table IV. We have calculated the average age in days when response was first observed to the various types of stimulus used. The last column in Table IV shows the average age in days when the first hearing

response was observed, irrespective of the type of the stimulus used. The first species to hear was cat, thereafter rabbit, dog and finally mink.

Results of ophthalmoscopy justify the con-

Table IV *Result of hearing test of all animals*

Species		Frequency of pure tones (Hz)								Type of noise		Stimulus producing the first hearing response
		125	250	500	750	1 000	2 000	4 000	8 000	Noise band 750 Hz	White noise	
Cat	\bar{A}	6.2	6.3	6.5	6.5	7.2	7.5	8.2	9.1	6.3	6.9	5.4
	s	2.1	1.4	1.4	1.4	1.3	1.1	1.1	1.3	1.2	1.5	1.9
	n	11	11	11	11	11	11	11	11	11	11	11
Rabbit	\bar{A}	7.7	7.0	6.8	6.7	8.5	8.3	9.2	9.7	6.8	7.7	6.7
	s	1.0	0.9	0.8	0.5	1.1	0.5	1.0	1.0	0.4	0.8	0.5
	n	11	11	6	6	6	6	6	6	6	6	6
Dog	\bar{A}	15.3	14.8	14.5	16.2	18.8	17.6	21.5	14.6	13.8	13.8	13.8
	s	0.6	0.5	1.3	2.5	4.8	4.2	1.0	0.9	0.8	0.8	0.8
	n	3	4	4	5	5	5	4	5	5	5	5
Mink	\bar{A}	34.3	30.0	30.2	34.0	35.0	38.5		31.5	31.2	29.2	29.2
	s	4.5	2.8	2.6	3.1	5.0	6.4		1.4	1.5	2.3	2.3
	n	6	6	11	6	4	2		8	6	6	6

 \bar{A} - Average age in days when response was first observed s - Standard deviation of \bar{A} n - Number of animals

clusion beginning of opening of eyelids corresponds with inception of visual function. The pupil variation was, however, far less pronounced than the orbicular reflex.

Fig 2 shows the correlation in occurrence of hearing and vision in the four test species. In Fig 3 the development of hearing in different frequency ranges is presented, together with opening of eyelids, as a function of age in the four test species. Weight as a function of age, together with the first observed auditory response and occurrence of vision in the four test species, are shown in Fig 4.

In Table V we have presented our results together with those reported by other investigators, in order to review the development of hearing and vision in various species as a function of age. For the same species a review is presented of the localization of frequencies along the basilar membrane, together with the frequency range in which the first auditory response was observed (see Fig 5).

DISCUSSION

Inception of hearing versus vision

In the four species studied by us it seems as if hearing occurs a little earlier than the opening of the eyelids. This is in general agreement with reports of similar work with other

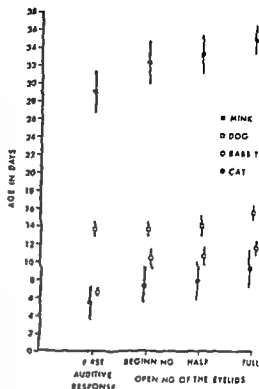


Fig 2 Age (arithmetic means) when first auditory response and when opening of the eyelids in the species were observed. Vertical lines indicate standard deviation (one sigma to each side).

species (Table V). The only exception to the rule is the guinea pig. The data for this species, however, is probably among the more uncertain, since opening of the eyelids is observed before birth. As a general rule it may

Table V Review of the development of hearing and vision in various species as a function of age

Species	Hearing development Behavioural response		Opening of the eyelids	
	Age	Reference	Age	Reference
Man (<i>Homo sapiens</i>)	24 weeks*	Johansson et al (1972)	24 weeks*	Contino (1907)
Guinea pig (<i>Cavia porcellus</i>)	5-6 hours	Wada (1923)	48-56 days*	Jasper et al (1937)
Cat (<i>Felis catus</i>)	5 days	Foss & Flottorp	7 days	Foss & Flottorp
Rabbit (<i>Oryctolagus cuniculus</i>)	7 days	Foss & Flottorp	10 days	Foss & Flottorp
Mouse (<i>Mus musculus</i>)	10 days	Mikaelian & Ruben (1965)	12-14 days	Erichsen (1969)
Rat (<i>Rattus norvegicus</i>)	10 days	Wada (1923)	12-14 days	Erichsen (1969)
Dog (<i>Canis familiaris</i>)	14 days	Foss & Flottorp	14 days	Foss & Flottorp
Mink (<i>Mustela vison</i>)	29 days	Foss & Flottorp	33 days	Foss & Flottorp
Opossum (<i>Didelphis virginiana</i>)	50 days	Larsell et al (1944)	50-60 days	McCrady (1938)

* Fetal age; therefore hearing and vision 16 weeks before birth.

* Fetal age; therefore vision 7-15 days before birth, assuming a gestational period of 111 days (Erichsen 1969).

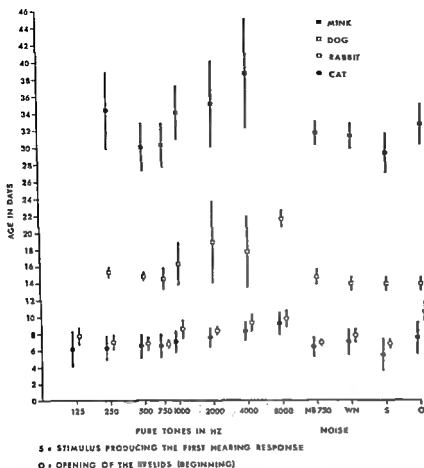


Fig 3 Hearing and vision (arithmetic means) in test species as a function of age. Vertical lines indicate standard deviation (one sigma to each side)

concluded that if one observes open eyes, i.e. full opening of the eyelids, the animal should hear, otherwise something is probably wrong with the hearing organ.

In addition to the method used by us (observation of the behavioural responses to sound), the function of a sense organ may be studied by recording the neural activity at various levels i.e. hearing by means of cochlear microphonics, action potentials or cortical responses, and vision in a similar manner (action potentials, cortical responses). It is conceivable that electrophysiological responses may be obtained before it is possible to observe behavioural responses. Based upon observations of several animals, however, we have reason to believe that the reflex mechanisms we have utilized were mature before the response to sound stimulation was ob-

served. Our method does not, however, allow a subtle evaluation of the maturation of hearing. The criterion used, some type of behaviour reflex involving the motor system cannot elucidate the hearing function for stimulus intensities below the reflex threshold—which is known to be rather high. The electrophysiological method should, therefore, be more sensitive and provide results indicating occurrence of hearing a little earlier than we have observed in the same species. The difference seems to be a constant factor of 2 days for hearing, and 2–3 days for vision (cat, rabbit, mouse, rat, opossum).

The middle ear may, however, lag in development. In mink we could observe auditive response before the middle ear was fully developed. In some electrophysiological studies the sound stimulus more or less bypasses the mid-

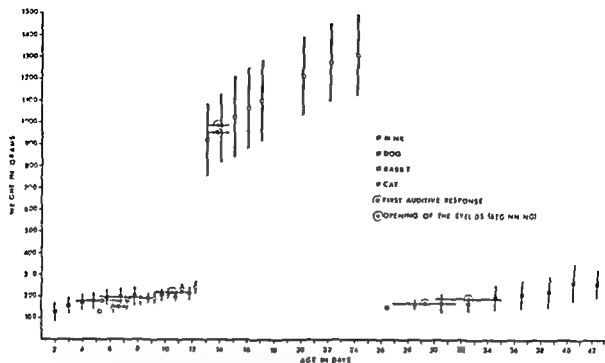


Fig. 4. Weight (arithmetic means) as a function of age, together with first observed auditive response and vision (arithmetic means) in cat, rabbit, dog and mink.

Vertical and horizontal lines indicate standard deviation (one sigma to each side)

dle ear, thus eliminating a possible attenuating factor of the unmaturing middle ear.

The possible selective influence of an unmaturing middle ear upon the transmission in different frequency bands has not been studied by us. We know, however, from clinical experience on humans that atresia of the ear canal (maximum loss 50–70 dB), fluid in the middle ear (maximum loss 30–40 dB) and similar middle ear pathology usually attenuate the entire auditive frequency range. We therefore do not consider that a possible lag in proper functioning of the middle ear should be responsible for a substantial delay of an observable auditive response to the sound intensities used.

In comparing our results with those reported in the literature it is also necessary to keep in mind the uncertainty about gestation length (date of conception, maturity at parturition), number of offspring per litter, possible differences between different breeds and between different animal husbandries as to feeding and care.

In addition there may be differences in definition of occurrence of hearing and vision. We have, for instance, defined our observations of opening of the eyelids in three categories and chosen "first observed opening" as our criterion for vision, whereas most researchers report nothing about their criteria. For hearing, the type of stimulus used is of great importance, since our findings show that hearing starts earlier for certain stimulus frequencies than for others.

In the following we will discuss in some detail results for our four examined species.

Rabbit

Of our animals the rabbit is the best defined. Our observations show that hearing occurs at the age of 7 days (Table IV). We have not been able to find any corresponding data in the literature, though Ånggård (1965) concludes from an electrophysiological study that hearing occurs at the age of 5 days. This is in good agreement with the earlier mentioned difference between results obtained using be-





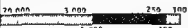






Species	Localisation of frequencies (Hz) number of coils and hearing development (frequencies for which response was observed localisation on  supposed localisation on )					References
	First coil	Second coil	Third coil	Fourth coil	Fifth coil	
Mouse	50 000 	1 420 2 000				Mikaelian et al (1965) Békésy (1960)
Opossum	26 000 	1 320 7 000	200			Larsell et al (1944) Larsell et al (1935)
Rat	20 000 	1 000 2 000	250 200			Crowley et al (1966) Békésy (1960)
Rabbit	20 000 		250 125			Duquesland (1907) Nabeys (1923) Foss and Flottorp
Man	20 000 	1 000 500	325	16		Békésy (1960) Bredberg (1968)
Cat						
Dog	50 000 		750 500		15	Stevens et al (1965) Asai (1908) Foss and Flottorp
Mink						Foss and Flottorp
Guinea pig	20 000 	3 200 1 000	300	180	62	Larsell et al (1944) Békésy (1960) Anderson et al (1965)
	First coil	Second coil	Third coil	Fourth coil	Fifth coil	

Fig 5 Review of the localization of frequencies along the basilar membrane in various species together with the frequency range in which the first auditory response was observed. The bars indicating the basilar

membranes are schematically drawn, giving each coil the same length. Therefore the figure has no resemblance to the physical dimensions.

Behavioural and electrophysiological responses

Opening of the eyelids, according to our definition and observation, occurs at the age of 10 days. Marty (1962) reported 9 days, and Erichsen (1969) 9–10 days.

Our observation of weight and its daily increase agrees with Venge (1953).

Cat

Our cats are of the usual mixed breeds with rather substantial variations in number of kittens in each of the four litters, and gestation length unknown. We observed rather large differences in age at time of occurrence of hearing and vision in the different litters. This was, however, not unexpected. Litter number one consisted of only 2 kittens, with

signs of overmaturity (at the age of 2 days weight 185 grams and hearing present, vision at the age of 4 days). According to Hall & Pierce (1934) the average litter size is 3.9, whereas the corresponding figure for our litters is 2.8. Our two litters of normal size (4) dominate the presented data; average age at inception of hearing being 5 days and for vision 7 days. Pujol et al (1967) and Pujol & Marty (1968) reported 2–3 days for hearing. Their results are, however, based upon electrophysiological measurements and fit therefore very well in the earlier-mentioned scheme of 2 days difference between electrophysiological and behavioural responses.

Marty (1962) and Erichsen (1969) report later occurrence of vision (9 days and 10 days)

than we do. The reason for this discrepancy may be different definitions of opening, or may be due to different sizes of litters and uncertainty of gestation period.

Our observation of weight and its daily increase agrees with Hall & Pierce (1934).

Dog

We examined 5 puppies and found that hearing occurred in an average of 14 days, coinciding with occurrence of vision. Based upon a study of 10 different breeds, Parry (1953) reports opening of eyelids at an age of 10–15 days, Erichsen (1969) 10–14 days. We have not been able to trace any corresponding hearing data in the literature. We are, however, convinced that a greater number of litters would provide results indicating the same small difference between occurrence of hearing and vision as reported for the other species. Our dogs had rectilinear weight increase (36 grams per day) equivalent to results for beagle (38 grams per day) (Andersen, 1970).

Mink

We conclude from our observations of 6 minks from three different litters that hearing occurs at an average age of 29 days, vision 4 days later. The age observed by us is distinctly greater than reported by Hilding et al (1967) 2 weeks and by Sugiura & Hilding (1970) 3 weeks. The difference between results may be due to the earlier mentioned variation in "real" gestation length, although different methods probably are the most important reason. The standard deviation for our mink data is rather great. We believe, however, they are reliable because we have corresponding results from examination of the white Hedlund mink. This species was tested in greater number of litters and animals (Flottorp & Foss, 1970, Foss & Flottorp, 1970, further details in a following publication).

As can be seen in Table V, we have observed opening of eyelids at an average age of 33 days (4 days later than inception of hearing). We have not been able to find cor-

Table VI Cochlear localization (human) for tones based on data from Bredberg (1968) and Bekesy (1960)

Coil	Distance from the basal end of the cochlea		Frequency (Hz)	Coil
	Angular measure (degrees)	Linear measure (mm)		
1st	0	0	(20 000)	1st
	12	3	10 000	
	35	5	8 000	
	60	7	6 500	
	120	10.5	4 200	
	135	11	4 000	
	170	13	3 000	
	180	13.5	2 800	
	215	15	2 400	
	255	16.5	2 000	
2nd	270	17	1 800	2nd
	360	21	1 000	
	425	23	750	
	505	25	500	
	540	26	430	
	560	26.5	400	
	600	27.5	250	
	625	28	200	
	720	30	125	
	745	30.5	100	
3rd	875	32.5	50	3rd
	1 030	34	(16)	

responding information in the literature. The reported age, resulting from our rather small number of animals, is exactly the same as observed by us in the just mentioned examination of Hedlund mink, comprising 18 kits. Our observation of weight and its daily increase agrees with results obtained at The Agricultural University of Norway during a period of 15 years from 3–4 000 mink kits (Rimeslåtten, 1973).

Frequency localization and maturity of hearing

Although our sound stimuli, from the weakest to the strongest, vary 15 dB in SPL, we consider them to be adequate for determining the frequency range first responded to. We gave the lower frequencies, for which the ear is usually a little less sensitive, the highest sound pressure level. The sound pressure level for all stimuli was very high, approaching

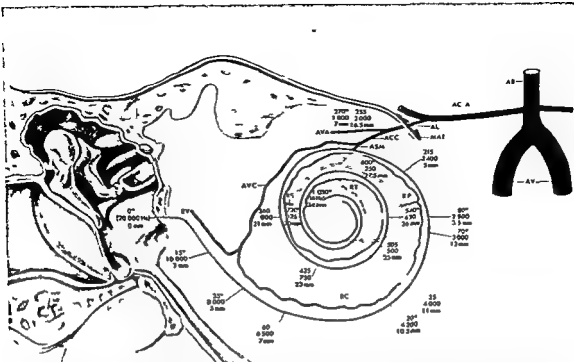


Fig 6 Human cochlea (anterior view of the right cochlea) blood supply and localization of frequencies. Schematically drawn from data extracted from Bredberg (1968) Bekésy (1960) Eichler (1892) Sebenmann (1894) Nabeya (1923) Scuderi & del Bo (1952) Charachon (1961) and Axelsson (1968). *AV* Arteria vertebralis *AB* Arteria basilaris *ACIA* Arteria cerebelli inferior anterior *AL* Arteria labyrinthi *AVA*

Arteria vestibuli anterior *ACC* Arteria cochleae communis *AVC* Arteria vestibulo-cochlearis *ASM* Arteria spiralis modiolus *RI* Ramus vestibularis (of *AVC*) *RC* Ramus cochlearis (of *AVC*) *RP* Ramus primus (of *ASM*) *RS* Ramus secundus (of *ASM*) *RT* Ramus tertius (of *ASM*) *MAI* Meatus acusticus internus

the pain limit (we used ear defenders during the test period)

According to Boothroyd & Cawkwell (1970) the possibility of tactile response to loud sounds below 500 Hz should be considered. Our animals however, did not respond to these low tones at an age for which the tactile sense without doubt was mature. In addition we have never observed the deaf Hedlund mink to respond to these low tones (study to be published later). The reported response to low frequency sounds in this investigation should therefore be genuine auditive.

The frequency range first responded to in all four species located to the second and third coil of the cochlea (see Fig 5). We consider this to reflect the status of development

of the cochlea. Although our method is rather coarse—only examining response to very high stimulus intensity—we find no reason to believe that the development of hearing shows any discontinuity. Thus the first observed response to high stimulus intensity in a certain frequency range only is considered to be indicative for the inception of hearing in the corresponding tonotopic location of the cochlea.

The retrocochlear part of the afferent system in particular the auditory cortex is probably a little ahead of the cochlea in maturation (Larsell et al, 1935; Marty, 1962; Chaloupka et al, 1968; Pujol & Marty, 1968). Therefore our study of the cochlear tonotopic localization should not be concealed by this part of the reflex arc. The middle ear may lag in maturity

as mentioned earlier, however, should not cause a significant change in response to different frequencies

It appears from data of other researchers that in the second and third coil of the cochlea—for some species also in the range between first and second coil—the organ of hearing first develops ability to respond to sound (for references see Fig 5) So far we can find no support for the oft advocated statement that hearing starts at the basal coil (see, e.g. Pujol et al, 1967)

The very interesting problem about a possible correlation between the tonotopic localization of frequencies first responded to and the topography of the blood supply to the cochlea, has been at the focus of our interest Our working hypothesis has been that the partition of the cochlea having the best blood supply will develop first and will be first matured to respond to acoustic stimulation

We realize that the ontogenetic development is not by chance governed by the presence or absence of sufficient blood supply Probably the pattern of development is genetically guided, however, the goal is reached by a careful layout of the vascular system, ensuring the organs and/or parts of organs wanted to be ahead in the development of the best blood supply

Since the most valid available data about the localization of frequencies along the basilar membrane and of the topography of the blood supply is for the human ear, we have tried to accumulate these data from different sources and combine them (see Table VI and Fig 6) From observations on the human fetus, Johansson (1972) suggests that the development of the cochlea starts around 1500 Hz and proceeds in both directions This conception fits very well with the topography of the blood supply to the cochlea (Fig 6)

The first branch (ramus primus, RP) of A spiralis modioli (ASM) is rather thin, whereas the second branch (ramus secundus, RS) is distinctly thicker (Siebenmann, 1894, Nabeya 1923) Therefore, the upper quart of the first

coil, together with the lower half of the second coil, i.e. the range from about 2000 Hz to 500 Hz, should have the best blood supply

On the other hand, the vestibular branch (ramus vestibularis, RV), although a rather thick branch, primarily destined for supplying the vestibular apparatus, gives off only tiny twigs to the basal area of the cochlea corresponding to frequencies above 9000 Hz (Axelsson, 1968) Especially the extreme basal end of the cochlea (caecum vestibulare) has very poor blood supply, being vascularized from a tiny recurrent branch (a radial section of stria vascularis contains only one capillary) (Axelsson, 1968) This fact may account for the observed vulnerability of hearing above 12000 Hz in man (Flottorp, 1973)

Anastomosis exists between A vestibulo-cochlearis (AVC) and A spiralis modioli (ASM) and also between the three branches (RP, RS and RT) of A spiralis modioli (Axelsson, 1968) This does not appear from the drawing in Fig 6, because we intended to show distinctly which parts of the cochlea were supplied from the various branches The anastomosis between AVC and ASM (at 4000 Hz) is poorly developed (Siebenmann, 1894, Nabeya, 1923, Axelsson, 1968) Clinical experience has for many years drawn attention to presbycusis and acoustic trauma causing hearing loss at the upper frequency range and around 4000 Hz, corresponding to the regions of poorer blood supply (Vard et al, 1959, Bredberg 1968, Flottorp 1973)

Unfortunately there is very little knowledge about hearing in man below 125 Hz, because this is the lowest frequency in most audiometers The localization of 125 Hz in cochlea is in the transition between the second and third coil Hearing corresponding to the apical coil is therefore almost never the object of clinical examination The blood supply to this part is rather poor, being the last place of irrigation of A spiralis modioli (ramus tertius, RT) According to Bredberg (1968) degeneration of the organ of Corti at the apical turn is found rather frequently

Consequently a close correlation seems to exist between the area in cochlea with abundant blood supply and the frequency range for which hearing first develops and which during degeneration persists longest. On the other hand, deterioration of hearing is found to start in the frequency range corresponding to regions with poor blood supply.

ACKNOWLEDGMENT

Mink kits were generously supplied by Assoc. Prof. Arne Helgebostad, Research Station for Fur-bearing Animals, The Veterinary College of Norway, Oslo, and Assoc. Prof. Hans Rimeslatten, Department of Poultry and Fur Animal Science, The Agricultural University of Norway. As Assoc. Prof. Martin Davanger, M.D., and University Lecturer Tor Flage, Eye Department, University Hospital, Rikshospitalet, Oslo, kindly performed the ophthalmoscopy.

ZUSAMMENFASSUNG

Die Aufnahme des Gehörfunktion und das Öffnen der Augenlider wurden bei Katze, Kaninchen, Hund und Nerz untersucht. Die Gehörteste wurden mit reinen Tönen und Lärmbanden durchgeführt, wobei man das Verhalten der verschiedenen Arten beobachtete. Es wurde festgestellt, dass das Gehör bei einem mittleren Lebensalter von 5 Tagen bei der Katze, von 7 Tagen beim Kaninchen, 14 Tagen beim Hund und 29 Tagen beim Nerz auftrat. Die Frequenzen auf die zuerst reagiert wurden und die nachfolgende Entwicklung des Gehörs für andere Frequenzen wurden angeführt und mit Daten in der Literatur für fünf andere Arten verglichen. Die Augenlider öffneten sich bei einem mittleren Alter von 7 Tagen bei der Katze, von 10 Tagen beim Kaninchen, 14 Tagen beim Hund und 33 Tagen beim Nerz. Der mögliche Zusammenhang zwischen Blutzufuhr zu den verschiedenen Wandungen der Schnecke (beim Menschen) und der ontogenetischen Entwicklung des Gehörs und der Herabsetzung des Gehörs im Alter und nach einem akustischen Trauma wurde erörtert.

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SURGICAL TREATMENT OF DROOLING

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Abstract Eleven persons with brain damage and embarrassing drooling have been operated on by a repositioning of the submandibular ducts combined with section and ligation of the sublingual ducts as a treatment against the drooling. Results of the treatment have been analysed by objective measurements and subjective judgments of drooling. Function studies of the submandibular glands have also been made pre and postoperatively. Nine of the 11 patients show by objective measurement of drooling a postoperative improvement. The subjective judgment often demonstrated diverging results by different nursing personnel within the same patient. No signs of postoperative atrophy were seen in the function studies of the submandibular glands.

Drooling is seen as a symptom in certain neurological diseases, e.g. Parkinson's disease, myasthenia gravis, cerebral palsy (CP) and is probably caused by hypersalivation and/or disturbances of the swallowing mechanism (Wilkie, 1967, Goode & Smith, 1970). Every year 200-300 children with CP are born in Sweden and about 10% of them will show an embarrassing drooling even after infancy (Enfors & Lundberg, 1968). This means that the contribution from this sector alone is 20-30 new cases a year. While drooling is also seen in other neurological diseases there must be several hundred individuals in this country who need to be treated against drooling.

Earlier studies on treatment of drooling have been few, covering a small number of patients and the estimation of the results has been made by subjective methods only. There is without doubt a considerable disproportion be-

tween the magnitude of the problem and the resources that have been used to help these individuals.

The therapy against drooling through the years has been anticholinergic drugs, mostly atropine. The drawback to this therapy is that the administered dose often has to be so high that unwished side effects are seen, e.g. loss of visual accommodation, headache, dryness of the eyes and urinary retention (Goode & Smith, 1970). As a consequence various methods of surgery have been tried, e.g. extirpation of the submandibular glands (Wilkie, 1967), repositioning of salivary gland ducts (Wilkie, 1967, Laage Hellman, 1969) and sectioning of the efferent parasympathetic nerves to the parotid, submandibular and sublingual glands (Yoel & Markman, 1963, Goode & Smith, 1970). Combinations of the different methods are also described (Enfors & Lundberg, 1968, Wilkie, 1967).

The surgical method described in this paper is a repositioning of the submandibular ducts combined with a ligation and division of the sublingual ducts. The technique is the same as that described by Laage Hellman (1969) except for the division of the sublingual ducts.

The purpose of the present investigation is

- 1 To estimate the results of surgical treatment of drooling by the help of the earlier described method for quantitative measurement of drooling (Ekedahl & Hallen, 1973)

- 2 To compare the author's quantitative

method with the subjective judgments made by the nursing personnel. The latter method corresponds to the estimations of the treatment results described in other articles (Voel & Markman 1963 Wikie 1967 Enfors & Lundberg 1968 Laage Hellman 1968 Goode & Smith 1970).

3 To ensure that a good postoperative result would not be caused by atrophy of the submandibular glands because the operative method used could result in kinking of the submandibular ducts with a possible secondary glandular atrophy.

MATERIAL AND METHODS

Eleven patients with brain damage mainly in the form of cerebral palsy and severe drooling were examined. The age distribution was 7–46 years, 5 female and 6 male. The intellectual capacity varied from idiocy to normal and 9 of the patients lived in an institution for mentally retarded. Pre and postoperatively the patients were examined with objective measurements and subjective judgments of drooling as well as function studies of the submandibular glands.

The patients were operated on under general anesthesia. Using a microscope an incision was made around the papilla of the submandibular duct which was then prepared from the surrounding tissues to the connection of the sublingual duct. In all the cases operated on one main duct from each sublingual gland was found. After ligation and sectioning of this duct the submandibular duct was prepared so that 2–3 cm were freely movable. With the help of a pair of scissors a submucous tunnel was made in the floor of the oral cavity backwards to the palatal arches where an incision was made. With a clamp the duct was brought through the tunnel and the papilla was fixed in the incision opening by a 4/0 atraumatic chromic gut suture. The whole procedure was then repeated on the other side whereafter the mucosal defect in the floor of

the mouth was sutured. Antibiotics were not given as a routine.

Objective measurement of drooling

Quantitative measurements of drooling were performed preoperatively and 1 year postoperatively. 100 μ Ci of the isotope ^{99m}Tc was given intravenously to the drooling patients. Saliva samples were taken every half hour 120–540 minutes after the isotope injection. The drooled saliva was collected every half hour between the saliva samples in disposable bibs (Molnlycke art 720500). The radioactivity in the saliva samples was determined in a spectrometer type Picker Autowell with a 3 inch well crystal of sodium iodide while the bibs were examined in a Packard Model 3007 TRI-CARB scintillation spectrometer and an Armac Model 440 scintillation detector. With the knowledge of radioactivity in the bibs and the saliva samples the amount of drooled saliva could be calculated. The method is described in detail in an earlier article by Ekedahl & Hallen (1973).

Subjective judgment of drooling

Every patient was judged preoperatively and 1 year postoperatively by 3 persons in the nursing staff with good knowledge of and experience from the nursing of each patient. The drooling was judged in three different situations: at rest, at mealtime and in an engaged situation, e.g. at play. In each situation it was graded as insignificant=1, moderate=2, rich=3 or very rich=4 units. The mean values from these judgments by the 3 persons of the nursing personnel were calculated for each situation and compared pre and postoperatively. A difference of more than 1 unit was classified as an improvement or impairment. From these differences the patients could be judged as free of drooling, improved, unimproved or impaired. Independent of this differentiated grading 3 persons of the nursing staff on another occasion postoperatively made an integrated judgment as to whether the patient was free of drooling, improved or unimproved or impaired.

Table I Results from pre- and postoperative objective measurements of drooled saliva and comparison with the results of the integrated subjective judgment

Patient	Age	Sex	Objective measurement				Integrated subjective judgment
			Preop ^a	Postop ^a	Difference ^b	% Improvement	
K A	10	♀	5.25	0.80	4.45*	84	Improved
C P	11	♀	2.11	0.18	1.93*	91	Improved
B J	11	♀	9.90	1.41	8.49*	85	Unimproved
L U	18	♀	9.09	2.33	6.69*	73	Improved
B O	19	♀	5.50	2.39	3.11*	56	Unimproved
A L	46	♀	3.92	0.28	3.64*	92	Improved
M S	7	♂	3.82	1.32	2.50*	65	Unimproved
P J	12	♂	3.05	2.95	0.10	3	Improved
J P	21	♂	4.30	0.76	3.54*	82	Improved
L H	21	♂	3.86	3.22	0.64	16	Unimproved
B K	26	♂	8.19	3.77	4.42*	53	Improved
Mean			5.35	1.76		64	

* Significance at a 5% level

^a Each value expressed in g/30 min is the mean of 42 measurements^b Difference between pre- and postoperative values

Control of function of submandibular glands

In order to exclude kinking of the retropositioned submandibular ducts, resulting in glandular atrophy, function of the glands has been controlled by scintigraphy. The patients got 0.25–0.50 mg atropine subcutaneously just before the administration of 200 μ Ci ^{99m}Tc in order to reduce the uptake of the isotope in the small acinary glands of the oral mucosa (Emmelin, 1967; Enfors et al., 1969). Twenty minutes after the technetium injection a scintigram was made with a Picker Magnascanner III with a 19 hole coarse focusing collimator. The scintigram was taken in the frontal position. Immediately after the scintigram had been taken, a quantitative measurement expressed in counts was made for 1 min over the submandibular glands with the patient in the same position (McG Harden et al., 1967). The same procedure was made 6–12 months after the operation and was compared with the preoperative measurement.

RESULTS

Objective measurement of drooling

The results of the pre- and postoperative measurements are shown in Table I. Because of

the earlier described variations in amounts of drooled saliva (Ekedahl & Hallén, 1973) 42 measurements preoperatively and an equal number postoperatively have been required to get an acceptable precision in the estimate of the mean. The comparison of the values is based on a *t* test with paired observations of the means from all the measurements or each patient. The statistical significance is tested at a 5% level. The results from the *t* test for the whole group shows *t* = 4.11 for 10 degrees of freedom, which means a difference between the pre- and postoperative results that is statistically significant. Examining each patient separately in the same way, differences pre- and postoperatively in 9 of 11 patients are statistically significant and in these 9 patients the percentage improvement varies from 53 to 91%.

Subjective judgment of drooling

The results from the differentiated and integrated judgments are shown in Table II. The differentiated judgments made by 3 persons of the nursing personnel in the 3 different situations show an improvement on all counts in only 2 of the 11 patients. There is a uniformity in the estimations in only 2 more cases, indicating no postoperative improvement. The

Table II Results from subjective estimation of drooling pre- and postoperatively

Patient	Differentiated judgment			Integrated judgment
	At rest	At mealtime	Engaged situation	
K A	Unimproved	Improved	Improved	Improved
C P	Improved	Improved	Improved	Improved
B J	Improved	Worsened	Improved	Unimproved
L U	Unimproved	Improved	Unimproved	Improved
B O	Unimproved	Unimproved	Unimproved	Unimproved
A L	Unimproved	Improved	Improved	Improved
M S	Unimproved	Unimproved	Unimproved	Unimproved
P J	Unimproved	Unimproved	Improved	Improved
J P	Improved	Worsened	Improved	Worsened
L S	Unimproved	Worsened	Improved	Unimproved
B K	Improved	Improved	Improved	Improved

remaining 7 patients generally show great variations in the differentiated judgment. Four and 5 patients respectively are improved at rest and at mealtime while 8 are considered to be improved in engaged situations. The integrated judgments made by 3 persons of the nursing staff with the purpose of getting a conclusive evaluation of the postoperative results show an improvement in 6 of the 11 cases. The differentiated and integrated judgments are in agreement only in those 4 patients who showed a uniformity in the differentiated judgment. The integrated subjective judgment is compared with the results from the quantitative measurement in Table I and there is a lack of coordination in not less than 5 of the patients.

Table III Isotope uptake (^{99m}Tc) in the submandibular glands pre- and postoperatively (expressed in cpm)

Patient	Preoperatively		Postoperatively	
	Right	Left	Right	Left
K A	8 951	9 138	4 894	5 901
C P	11 265	9 841	8 135	7 913
B J	3 514	3 076	3 951	3 432
L U	4 526	5 320	3 349	5 471
B O	5 499	4 668	4 839	5 201
A L	2 159	2 337	5 246	5 508
M S	6 732	6 009	9 163	9 503
P J	9 596	10 012	5 303	8 841
J P	3 445	3 594	5 221	5 722
L S	3 304	3 245	6 869	6 855
B K	3 710	4 174	4 282	4 694

Function of the submandibular glands

The results from the isotope uptake measurements of ^{99m}Tc in the submandibular glands are seen in Table III. The time interval between the operation and the postoperative scintigraphy is 6-11 months (mean 10 months). In 10 of the 11 cases no reduction is seen in the postoperative values compared with the preoperative. In case K A a postoperative reduction is seen in both glands which probably is the result of different functional states. This seems still more probable as many of the patients show higher postoperative count rates than the preoperative ones. The background activity has also been tested in some of the patients directly after the scan had been taken and the figures did not exceed 1 100 cpm. Thus the results speak in favour of a good function of the submandibular glands in the patient K A.

DISCUSSION

The probability of a combination of several etiological factors in cases of drooling makes the choice of therapy difficult. Irradiation of the salivary glands should be avoided on these indications. The medical therapy has been transcribed because of the disadvantage earlier described in this paper and furthermore many of the 11 patients have already been treated with anticholinergics mostly without success. The aim of the author has been to use a surgical method which is minimally destructive. As

Wilkie (1967) showed a disturbance in the saliva transport from the frontal parts of the mouth to the pharynx in children with cerebral palsy and drooling, it seemed wise to try to overcome this problem by letting the saliva from the submandibular ducts fall directly into the pharynx. Another reason for using the actual surgical method is that most of the drooling occurs during a state of 'resting secretion', which in 60-70% comes from the submandibular and sublingual glands (Schneyer & Levin, 1955, Rauch, 1959). For the above-named reasons the patients have been operated on with a bilateral retropositioning of the submandibular ducts combined with a ligation and sectioning of the main ducts to the sublingual glands.

No complications were seen during the operations. In 1 case a postoperative haematoma was seen in the floor of the mouth causing slight, transitory respiratory problems. Fluids or mashed food could generally be accepted already during the first postoperative day and the patients could be sent to their homes or nursing homes 1-2 days after the operation. In no case was a distressing swelling of the submandibular or sublingual glands to be seen.

The study shows that 9 of the 11 patients are improved by the operation, as measured by the objective method. The one of the unimproved patients (L S) has a poor intellectual faculty, a spastic tetraplegia and marked difficulties in swallowing as shown in an earlier article (Ekedahl et al, 1973). The patient was described by the personnel postoperatively as unimproved.

The second patient (P J) has a spastic diplegia, is mentally very poor but has no serious disturbance of the swallowing mechanism. This patient almost always sits bent forward with his mouth open, which probably explains the poor result of the operation. Both of the unimproved patients were treated with benzodiazepines which, according to Hagberg (1968) and Eeg Olofsson (1973), could result in hyper salivation.

In the subjective differentiated judgment the study often shows divergent estimations of drooled saliva by different nursing personnel within the same patient. The integrated judgment also shows great differences in the estimated treatment results, as reported by various members of the nursing staff. Apart from these facts only a weak correlation is seen between the differentiated and integrated judgments. The results indicate a marked uncertainty concerning the subjective measurement of drooling which thus could not possibly constitute a basis for comparison of the results from different kinds of treatment.

It has been questioned if the retropositioning of a salivary gland duct could result in kinking of the ducts and if so, will the result be a glandular atrophy. If the kinking results in total obliteration of the salivary gland duct, a glandular atrophy would be expected. This opinion is supported by the fact that ligation of salivary gland ducts in rabbits and mice has resulted in histologically demonstrable glandular atrophy (Wallenborn et al, 1968, Junqueira, 1951, Standish & Shafer, 1957, Tamarin, 1967). After ligation of the duct of Stensen in a patient, Baron & Ober (1962) found atrophy of both the tubular and acinar cells in the treated gland.

The uptake of ^{99m}Tc is presumed to be reduced in an atrophied gland as in chronic sialoadenitis and Sjogren's syndrome with known salivary gland atrophy, where a reduced glandular uptake of ^{99m}Tc has been shown (Fridrich & Wey, 1968, Grove & di Chiro, 1968, Schall et al, 1971). In the present study, there was, however, no reduced uptake in the submandibular glands 6-11 months after the operation, which must support a conclusion that the operative method used with retropositioning of the submandibular ducts has not resulted in kinking of the ducts, thus causing an atrophy of the glands.

ZUSAMMENFASSUNG

Elf Personen mit Gehirnschaden und belastigendem Geifer sind durch eine Retroposition der Ausführungsgänge der Submandibulardrüsen operiert worden kombiniert mit einer Ligatur und Abschneidung der Ausführungsgänge der Sublingualdrüsen als Behandlung gegen den Geifer. Die Resultate der Behandlung sind im Zusammenhang mit prä- und postoperativen Untersuchungen einschliesslich objektiver Messung bzw. subjektiven Schätzungen des Geifers und der Funktion der Submandibulardrüsen analysiert worden. Neun der elf Patienten weisen bei objektiver Messung des Geifers eine postoperative Verbesserung auf. Die subjektiven Schätzungen von verschiedenem Behandlungspersonal bei denselben Patienten ergaben oft divergierende Resultate. Keine Zeichen von postoperativer Atrophie konnten in den funktionellen Untersuchungen der Submandibulardrüsen beobachtet werden.

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IDIOPATHIC HEMIFACIAL SPASM

New Method of Surgical Treatment

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Abstract A new surgical technique is presented for the treatment of the idiopathic hemifacial spasm. It consists in exposing the nerve in the third portion of the Fallopian canal performing a longitudinal neurotomy and inserting a silicon rubber sheet through it to avoid nerve regeneration. Comments are made concerning the etiology diagnosis and surgery of this disease. Three cases are presented along with the results obtained.

In the idiopathic hemifacial spasm one side of the face is agitated by paroxysmal clonic involuntary and episodic muscular contractions usually very noticeable in the lower eyelid and labial commissure. This causes an unpleasant grimace that is aggravated under emotional tension making the patient feel miserable when being inquisitively watched by third persons.

The affection must be clinically differentiated from the post paralytic hemifacial spasm (frequent sequelae of severe Bell's facial palsy not opportunely decompressed) and from hemifacial spasm symptomatic of a compressive lesion of the facial nerve usually tumorous and located in the cerebello pontine angle or temporal bone.

Since it does not respond to medical therapy many surgical methods have been described for its treatment but none has been generally accepted. In this article we will discuss the etiology of the disease its diagnosis and the different operative techniques that have been developed. We will further propose a new technique that we hope will be useful in controlling this antieesthetic affection.

The etiology of idiopathic hemifacial spasm is unknown. There are several relevant theories

Otologic thesis

Many authors (Woltman et al 1951 Williams et al 1952 Proud 1953 Pulec 1972) believe the causative lesion is an edema of the facial nerve or a fibrous constriction of its sheath somewhere along its course through the Fallopian canal.

Neurosurgical thesis

The lesion could be caused by continuous and prolonged slight pressure upon the seventh nerve by a vascular structure in the posterior cranial fossa (Gardner & Sava 1962 Janetta 1972).

Neurological thesis

The pathological condition could be located in the motor nucleus of the facial nerve in the brain stem (Wartenberg 1952 Cruet et al 1968 Liebalt & Henn 1970).

Authors report dissimilar findings in defending these various theories each submitting positive proof in support of his ideas. This suggests that idiopathic hemifacial spasm does not have a unique cause but that in different cases it may have different etiologies. Clinically it is very difficult if not impossible to determine in a patient with that disease whether the lesion is located in the nucleus in the cerebello-pontine angle or in the Fallopian canal. This circumstance must be taken into account when selecting a surgical technique.

Physiopathology of this disease is better known. A series of involuntary anomalous and

frequent, motor impulses travel through the facial nerve. They may be electromyographically registered in the facial muscles and have well known characteristics (Isch & Jesel, 1967).

Supporters of the nuclear thesis believe these impulses originate in the ganglionic cells of the facial nerve in the brain stem, disturbances in the excitability threshold of those cells have been reported (Liebalt & Henn, 1970).

Those who accept the peripheral etiologic hypothesis postulate the existence of an irritating focus in any segment of the affected facial nerve. This area behaves as an artificial synapse transforming the presynaptic orthodromic impulse into a series of postsynaptic impulses, reflecting the antidromic impulses in the fashion of a pseudoreflex, and "short-circuiting" all nerve impulses reaching the area to the adjacent nervous fibers (Isch & Jessel, 1967).

The operative techniques that have been developed can be classified in two groups. One designed to act on the disease etiology, and the other, on its physiopathology.

Those who favor the first technique, the supporters of the otologic thesis, perform a decompression of the facial nerve incising its perineurium.

Initially, only the mastoid portion of the facial canal used to be decompressed (Woltman et al., 1951, Proud, 1953), later, as the otoneurosurgical techniques developed, the entire facial nerve was decompressed from the internal auditory canal to the stylomastoid foramen (Pulec, 1972). The majority of these authors report finding abnormalities such as edema, fibrous constriction of the perineurium, or diffuse thickening of it. However, Cawthorne (1965), in thirteen cases, found no alternations of that nature. Apparently, the results of this procedure depend upon the surgical traumatism on the nerve, the best results being obtained when the postoperative facial paralysis is more severe and long lasting.

Supporters of the neurosurgical thesis manipulate the nerve in the posterior cranial fossa by the suboccipital approach. In a remarkably high number of cases they have reported com-

pression of the nerve by a vascular structure: carotid aneurysm of the basilar artery, arteriovenous malformation, loop of the anterior-inferior cerebellar artery (Laine & Nayrac, 1948, Gardner & Sava, 1962, Janetta, 1972). They manipulate the nerve in the cerebello-pontine angle, separating it from the vessel that may be eventually compressing it. In using this technique it is possible to injure other nervous structures especially the eighth nerve, sometimes causing irreversible alterations. Furthermore, it is difficult to see how it is possible to separate permanently a vascular structure of the cerebellar pontine angle from the seventh nerve as its manipulation is delicate, and could cause the patient's death.

The otologists' findings, as well as the neurosurgeons', are strictly subjective, being most remarkable for the great contradictions existing between them.

As it is impossible in these cases to establish the etiology preoperatively, other techniques tend to direct the treatment towards the physiopathology of the disease, i.e. to diminish the number of axons that transmit motor impulses which are abnormal, involuntary, frequent and of unknown origin.

One of the oldest and most commonly used methods was alcohol injection of the nerve where it leaves the stylomastoid foramen (Harris & Dickson, 1932). This procedure has been recently modified by Wakasugi (1972) who traumatizes the nerve by inserting a needle in the facial trunk at the same point. These methods produce a transitory facial paralysis and therefore, absence of the spasm during the time the same lasts. But they lead inevitably to recurrence since they permit nervous regeneration, and what is worse, aggravate the spasm by creating a new irritating focus. New artificial synapses are caused and also misdirection of the regenerated fibers.

Another group of authors has developed techniques to partially and definitively decrease the number of axons, thus preventing its regeneration. Some surgeons approach the nerve in the parotid region, beyond its bifurcation,

performing longitudinal hemisections in the various branches. Afterwards, they fold the sectioned fragment centrally, tying it to its trunk of origin (Miehke, 1961, Diamant et al, 1967). Similar procedures have been devised for blepharospasm, except that these make a selective, definitive neurotomy instead (Reynolds et al, 1967, Harvey, 1971, McCabe & Boles, 1972). These techniques leave unsightly scars, and in case of recurrence, make it very difficult to reoperate by the same approach, since the cicatrization hinders the dissection of these thin branches (Diamant et al, 1967).

Analogous techniques have been described approaching the nerve in the mastoid process and performing longitudinal partial neurotomies. The sectioned portion is folded centrally (Scoville, 1965), or buried in openings made, utilizing a burr (Lewis, 1965), in the mastoid bone in order to prevent regeneration.

In this paper we present a new, simple and practical method that we have devised along these lines.

MATERIAL AND METHODS

Diagnosis

Clinical characteristics of this disease have been described at length (Ehni & Woltman, 1945). We will only emphasize the parameters we apply to classify a spasm as idiopathic.

(a) Absence of other otoneurologic symptomatology (excluding tinnitus and/or fugacious vertigo, since these may be present in any type of hemifacial spasm). They are caused by contractions of the stapedius muscle, simultaneous with the spasm).

(b) Normal temporal bones X-ray study.

(c) Normal posterior fossa X-ray study (should include at least myelencephalography).

(d) Normal audiologic and electronystagmographic study.

(e) Absence of history of homolateral peripheral facial paralysis.

The need to make all these tests is obvious. Any hemifacial spasm may be symptomatic of an

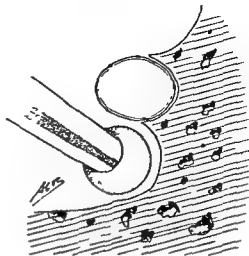


Fig. 1 Dissection of the facial nerve utilizing the burr. Exposing the nerve 270°, lateral and posterior surfaces and slightly more than 50% of its medial surface.

expanding lesion in the cerebello-pontine angle or of a tumor in the temporal bone.

Technique

To gain access to the third portion of the facial nerve, we use the transmastoid approach following the usual procedure of decompressing this portion of the nerve (Lewis, 1957). This technique is so well known that we will not describe it in detail.

Mastoidectomy should be ample, skeletonizing the lateral sinus and thoroughly exposing the fossa incudis and the digastric ridge, thus providing more room and allowing better maneuvering at the facial nerve level.

The seventh nerve is then skeletonized by using a cutting burr initially, and later, removing the last osseous layers with a diamond burr. Thus, only a fine membrane is left covering the nerve and which can be later removed with a small hook. Continuous suction irrigation should always be used. Tympanotomy at the facial recess should be performed between the chorda tympani and the Fallopian canal in order to fully expose the third portion.

The removal of bone around the facial nerve is performed in an extension of approximately 270° exposing very well the lateral, posterior, and

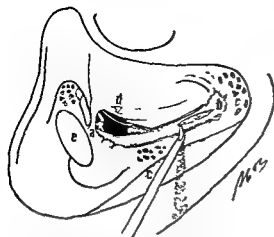


Fig 2 Incision of the perineurium (a) Fossa incundis (b) digastric ridge, (c) lateral sinus, (d) posterior tympanotomy, (e) horizontal semicircular canal

medial surfaces of the nerve trunk. The latter portion should be exposed by over 50% to permit performing the neurotomy (Fig 1).

Once the nerve is exposed, the perineurium is incised all along the third portion (Fig 2). Later, using a thin and very sharp knife, the neurotomy is performed dividing the nerve in half along its major axis (Fig 3). The extension of the incision is directly proportional to the spasm's severity. After concluding the neurotomy, a very thin sheet of silicon rubber, of 0.12 mm gauge, is inserted in its sinus, slipping it gently between the two nerve trunk halves and folding its lower end backwards to prevent it from slipping out of the neurotomy (Figs 4 and 5). The length of this sheet depends upon

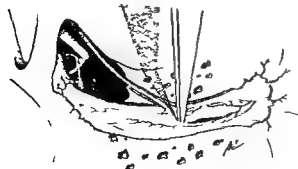


Fig 3 Longitudinal medial incision of the facial nerve along its major axis



Fig 4 Silicon rubber sheet inserted in the neurotomy

the size of the neurotomy and its width is of 1 cm.

Clinical cases

We have previously reported the methods used in exploring the facial nerve (Celis-Blaubach 1968).

Case 1

A 62 year-old woman developed a right hemifacial spasm 1 year before examination. No other symptoms were present. Physical examination of the nose, throat, and ears was entirely normal, as was a neurological examination. The

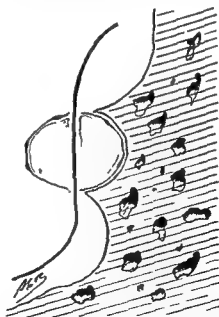


Fig 5 Longitudinal section of the nerve, showing the position of the silicon rubber sheet between the two halves of the nerve

tear test showed 50 mm of saturation in 5 minutes on the right side, and 50 mm in 8 minutes on the left. The stapedial reflex was absent on the right side. Electric taste tests revealed a threshold of 38 μ A on each side, and chemical gustometry was within normal limits. Excitability test showed a threshold of 4 mA on each side. Audiometry, tympanometry and electronystagmography were normal. Plain X-rays of the temporal bone and positive contrast myelography of the posterior fossa, showed no abnormality. On October 15th, 1970, surgery, following the procedure here reported, was performed. No edema or alterations of the facial nerve sheath were found. A neurotomy of 5 mm was done. Facial paresis appeared immediately after surgery. After 1 month, facial motility was almost normal. Six months following surgery slight fibrillar muscular contractions appeared on the right lower lid. Two years after the operation these were still present, but had not worsened.

Case 2

A 58-year-old woman, had left hemifacial spasm of progressively increasing severity for 6 years. No other complaints were presented. Physical examination of the nose, throat and ears was within normal limits. Neurological examination was also normal. Schummer's tests revealed 50 mm of saturation in 10 minutes on the right side and 50 mm, but in 90 seconds, on the left. Stapedial reflex was present on both sides. Electrogustometry showed a threshold of 50 μ A on each side and the gustogram was normal. Excitability test revealed a threshold of 5 mA on the right side, and of 6 mA on the left. Audiometry, tympanometry and electronystagmography showed no abnormalities. Plain X-ray and positive contrast roentgenography of the posterior fossa, were normal. On April 1st, 1971 surgery following the technique here described was performed. Facial nerve showed no alteration. Since the spasm was extremely severe, an extensive neurotomy (all along the mastoid portion of the facial nerve) was performed.

Severe facial paralysis appeared immediately after surgery. Two months later, first voluntary facial movements began to appear, and 7 months later, only moderate facial paresis persisted, visible when making extreme grimaces. Seventeen months postoperatively there was not the slightest sign of recurrence.

Case 3

A 22-year old woman complained of left hemifacial spasm of 20 months' duration. Since the onset this symptom was accompanied by left tinnitus and occasional vertigo, simultaneous with the spasmodic crisis. Physical examination of the nose, throat and ears, were within normal limits. Neurologic examination revealed a moderate decrease of the cutaneous sensibility in the left fifth nerve area. Nevertheless, left corneal reflex was normal. The tear tests were symmetric. The stapedial reflex was absent on the left side. Electric taste tests revealed a threshold of 64 μ A on the right side and 8 μ A on the left. Gustogram was normal. Audiometry, tympanometry and electronystagmography were within normal limits. Plain X-rays of the temporal bones and the cervical column, and a left vertebral and carotid arteriography, were normal. Pneumoencephalography showed a slight filling defect in the left cerebello-pontine cistern, but myelencephalography and brain scanning were normal. On June 11th, 1970, a left facial nerve decompression, utilizing the mastoid approach, was performed. Perineurium was incised and several small vertical neurotomies were made in the facial nerve (Portmann, M., 1968). Slight facial paresis appeared immediately after surgery and the spasm disappeared. Only small fasciculations on the left lower lid were noticeable. One year after surgery the hemifacial spasm recurred being even more severe. On August 3rd, 1972 a second operation, following the procedure we described in this paper, was carried out. A 1 cm neurotomy was made. The spasm disappeared and the postoperative facial palsy lasted 2 months. Six months after surgery she was feeling well, without evidence of spasm.



Fig. 4 With a pair of scissors or hemostats the instrument can easily be angled to a desired shape

angle on a cutting instrument would be helpful. By using an injection needle on this handle and a pair of scissors, or hemostats or forceps, a sharp instrument of most desired angles and shapes can easily be produced.

ZUSAMMENFASSUNG

Der Sauger im HNO-Dienst ist zum Saugen sowie als ein mechanisches Instrument zu verwenden. Dies stellt besondere Anforderungen an die Stärke des Saugers.

Heute können nur Metallsauger für mehrmaligen Gebrauch diesen Anforderungen entsprechen. Trotz sorgfältiger Reinigung blockiert der Sauger im allgemeinen nach wiederholter Verwendung. Um den Anforderungen zu entsprechen und die Nachteile auszuweichen ist ein Metallsauger für einmaligen Gebrauch entwickelt worden.

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ULTRASTRUCTURE OF THE COCHLEAR BLOOD VESSELS

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Abstract The ultrastructure of the cochlear blood vessels from the artery to the vein has been investigated. Some major differences between the small vessels of the modiolus and those of the membranous labyrinth are noted. Morphological evidence suggests that the major control of blood flow to the organ of Corti is accomplished at the modiolus. Fenestrated capillaries are seen among the modiolar cells which are unusually rich in cell organelles. These vessels are probably the major source of modiolar fluid and may be engaged in reduction of blood pressure and pulsatile movement of the blood vessels. Intracellular filaments in bundle form are common in the endothelial cells of the artery, arterioles and the arterio-venous arcades. Their presence is associated with vessels in which blood flow is very fast. Capillaries of the stria vascularis come directly in contact with marginal, intermediate and basal cells. The venules and inferior cochlear vein are surrounded by widely spaced pericytes or primitive smooth muscle cells and scant fibrocytes. Nerve fibers are lacking adjacent to the vessels of the membranous labyrinth with the exception of the tympanic lip of the limbus spiralis.

The ultrastructure of blood vessels in various organs has been described in great detail and reviewed by numerous investigators (Bennett et al, 1959, Farquhar, 1961, Palade, 1961, Fawcett, 1963, Luft, 1965, Majno, 1965, Rhodin, 1968). In the inner ear some fine structural studies of blood vessels have been reported by Smith (1957), Hinojosa & Rodriguez Echandia (1966), Hawkins (1967), Iurato (1967), Kimura & Schuknecht (1970), and Duvall et al (1971). More recently, a comparative study of the labyrinthine vessels of

different species has been reported by Wersall et al (1973). The present study is intended to provide a full description of the entire vascular system of the cochlea from the arterial to the venous sides indicating morphological differences along the course of the vessels.

MATERIALS AND METHODS

Thirty-three guinea pigs weighing 300 to 400 grams were used for this study. The specimens were fixed in 1% phosphate-buffered osmium and others were fixed initially with Karnovsky's fixative (1965) and post-fixed with osmium. The fixatives were perfused through the opened oval and round windows. For the modiolar vessels, the fixatives were pumped in from the internal auditory canal after the apical part of the cochlea was opened. The blood vessels encased in bone were prepared both undecalcified or decalcified in 10% EDTA. The specimens were embedded in Epon, cut with an LKB ultratome, double stained with uranyl acetate and lead citrate, and examined with a Siemens Elmiskop I at magnifications from 1000 to 40000 \times . The diameter of the vessels is recorded from mid point to mid point of the endothelial cells.

FINDINGS

The cochlear artery is a branch of the inferior cerebellar artery and ascends toward



Fig 1 Main cochlear artery showing endothelial cells (E) elastic layer (EL) smooth muscle cells (S), and adventitia composed of fibrocytes (F) and collagen

fibers (C) Note unmyelinated nerve fibers (arrow) $\times 6000$

the apex, spiralling around the cochlear nerve trunk. The artery is about 83μ at the base of the modiolus and becomes gradually smaller as tributaries are sent out toward the membranous labyrinth. The arterial wall is composed of endothelial cells, an elastic layer, one to three layers of smooth muscle cells and adventitia (Fig 1). The endothelial cell is tall (2 to 4μ) and contains an enormous number of filaments (67 \AA in diameter). Mitochondria and rough endoplasmic reticula are few, but many RNA particles are recognized. A few light and dense oval masses and multivesiculated bodies are also noted. Pinocytotic vesicles are small in number and are located mostly at the basal surface. At the luminal surface marginal folds and a few microvilli are observed. The profile of the cell junction is often straight, vertical to the cell surface, but other forms such as straight slant,

C, S, or Y types of interlockings are also seen. The tight junction is often long or intermittent. Another type of junction is the simple abutment of plasma membranes with condensation of adjacent cytoplasm.

The basement membrane of the cochlear artery is continuous except in areas where the smooth muscle cells establish a tight junction with the endothelial cells, and where intercellular fibrils abut endothelial cells. It extends outward into the elastic layer in a honeycomb arrangement. The elastic layer is about 1μ in thickness and is interspersed with fibrils or tubules (127 \AA) which follow the extension of the basement membrane. Collagen fibers (427 \AA) are sparse on the endothelial side but numerous at the periphery of the smooth muscle layer. Unmyelinated nerve fibers are common and contain many vesicles but vesicles with a core are rarely seen. The

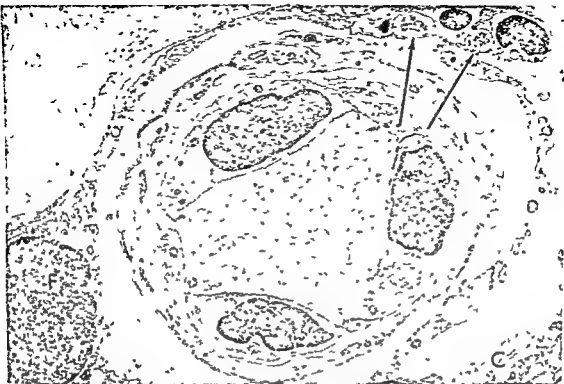


Fig 2 An arteriole (8.8μ diameter) of the modiolus. Smooth muscle cells completely surround the endothelial cells. Outside the vessels are patches of col-

lagen fibers (C), veils of fibrocytes (F) and a few unmyelinated nerve fibers (arrow) surrounded by a Schwann cell $\times 7000$

nerve fibers come close to the smooth muscle, but are never seen attached to it. Outside the collagenous layer are fibrocytes which form a series of thin cell processes (veils), in as many as seven layers, following the curvature of the vessel. Melanocytes are frequently seen around the arteries. Their cell processes appear to touch the basement membrane of the smooth muscle cells. Presumably, they are involved in phagocytosis.

Before leaving the modiolus for the membranous labyrinth, the smaller cochlear arteries and arterioles twist and loop within the stroma of connective tissue cells. These vessels retain distinct smooth muscle cells down to the size of 5μ (Fig 2). In most cases the vessels are obviously contracted as evidenced by a wavy basal surface. The most prominent feature of the arteriolar endothelial cell is the presence of numerous filament bundles. Other cell organelles are simi-

lar to those of the larger arteries. Pinocytotic vesicles are located more on the basal surface. The elastic layer becomes thinner but is still recognizable down to the size of 7μ . The smooth muscle layer is thin and dense foci within the cytoplasm become exceedingly difficult to visualize. The smooth muscle makes contact with the endothelial cell by extending small cell processes to the flat cell surface or to the invagination, also, the endothelial cells send processes to the smooth muscle cells. The adventitia is similar to that of larger arteries but the number of fibrocytes and collagen fibers is greatly decreased. Unmyelinated nerve fibers are found in the area (distance about 0.2μ) adjacent to the arterioles up to 5μ in size, they are partially or completely surrounded by Schwann cells (Fig 2).

An unexpected finding is fenestrated vessels in the modiolar connective tissue (Figs 3 A, 4). Their diameter is about 5.3μ , and



Fig. 3. (A) Coiled vessels of the modiolus surrounded by a series of the attenuated cytoplasm of modiolar cells (M). The vessel at the upper left corner is a fenestrated type. $\times 5000$. (B) Unmyelinated nerve fi-

bers (arrow) are surrounded by concentrically arranged cell processes of modiolar cells (M) at some distance away from the blood vessels $\times 8000$.

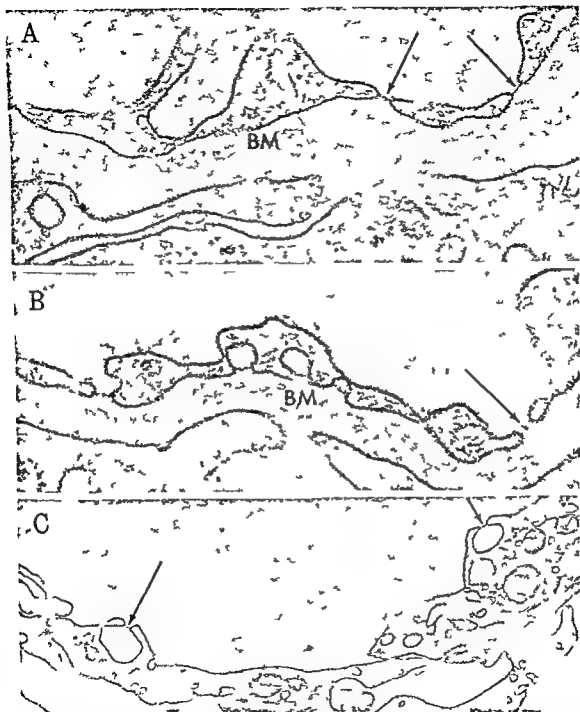


Fig. 4 (A) A fenestrated portion (arrows) of the modiolary capillary endothelium. The fenestra measures about 550 Å across. Basement membrane (BM) $\times 55\,600$. (B) A fenestrated capillary of the modiolus. One of the fenestrae appears to contain a central knob (arrow). The pinocytotic vesicles show an at

tenuated membrane across the opening toward the basement membrane (BM) $\times 65\,200$. (C) Fenestrated capillary of the modiolus. The attenuated membranes (arrows) are shown on the luminal side of the vacuoles which contain a substance similar to that of the capillary lumen $\times 32\,700$.

they are rather frequently seen along with other non fenestrated capillaries and arterioles. They have a distinct feature of overall irregular thickness of the endothelium and are marked by the presence of tall microvilli and polyp like projections into the lumen (Fig 3 A). The fenestra are in the magnitude of 550 Å in diameter. Some of the pinocytotic vesicles on the basal surface retain the attenuated membrane (Fig 4 B). Attenuated fenestra are also seen on the luminal side of the endothelium covering the large vacuoles (Fig 4 C). The endothelial cells do not contain filaments in bundle form. They are rich in RNA and some of the rough endoplasmic reticula are dilated. Vacuoles are frequently seen and pinocytotic vesicles are present in moderate number, more on the peripheral side even near the fenestra. The basal surface is completely covered by the continuous basement membrane which averages 1100 Å (range 990 to 1270 Å) when measured from the outer margin of the dense part to the endothelial cell. Pericytes make contact with the endothelial cell but do not extend over the fenestra. The vessels are surrounded by a thin layer of fibrils and by the modiolar cells. A preliminary study of the blood vessel within the cochlear nerve trunk shows that it is not fenestrated.

The loose connective tissue of the modiolus is composed of blood vessels, fibrocytes, unmyelinated nerve fibers, collagen fibers and fibrils. The fibrocytes which constitute a major part of the tissue are different from those seen surrounding the major arteries. These cells, modiolar cells, are very rich in large mitochondria, coated vesicles, and contain prominent large Golgi apparatus, many dilated rough endoplasmic reticula and numerous RNA particles in rosette form or in spiral, string like arrangement (Fig 5). Their nuclei are oval and large, and one or two centrioles or even short kinocilium may be seen near the nucleus. The cytoplasm contains diffuse filaments and oval masses and sometimes rod shaped crystalloids (Fig 5 A). The main cell

body often lies close to the fenestrated vessels, but more often their cell processes are attenuated and stacked in parallel where fascia occludens as well as desmosomes are frequently recognized. Some of the modiolar cells appear in different phases of activity, their cytoplasm contains many less granulated endoplasmic reticula, large dense bodies and the Golgi network is less conspicuous (Fig 5 B). Unmyelinated nerve bundles are found in the midst of the modiolar cells away from the blood vessels (Fig 3 B), the presence of both adrenergic and cholinergic nerve fibers in the modiolus was reported recently by Wersall et al (1973).

The cochlear vessels after leaving the modiolar connective tissue enter into another twisting within the modiolar bone. The arterioles seen in the bony channel are different from those seen among the modiolar cells. The arterioles (16 μ) are rarely constricted to the degree seen in the modiolus. The typical morphological appearance of the smooth muscle is less evident. They lack the elastic layer as well as nerve fibers, however, nerve fibers are recognized by a fluorescence method described by Terayama et al (1966). The endothelial cells become flat, though some bulge is noted toward the lumen. The cell organelles are decreased in number, but filaments in bundle form are clearly recognizable. In the adventitia collagen fibers are not recognized and are occupied by densely packed fibrils and by a few fibrocytes.

The arterioles in the bony interscalae partition and in the spiral ligament adjacent to the scala vestibuli are smaller but similar to those seen in the modiolar bone. Thin smooth muscle cells or pericytes partly or completely surround the endothelial cells (cell height 0.3 μ) (Fig 6). The difference between smooth muscle cells and pericytes becomes less distinct as the size of the vessels is reduced. In general, pericytes are thinner and do not surround the endothelial cells as much as the smooth muscle cells do, the dense foci at the central part of the cytoplasm are less con-

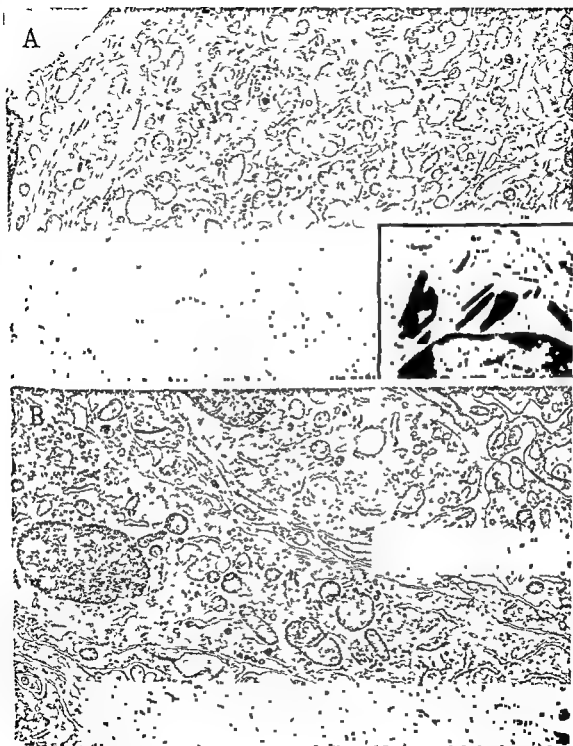


FIG. 5 (A) Perivascular modiolar cells. The cells contain numerous large mitochondria and the cell processes interdigitate considerably $\times 11\,000$. The inset shows modiolar cells containing elongated crystalline substances and desmosomes between adjacent cells

$\times 14\,300$ (B) The modiolar cells showing elongated and less granulated endoplasmic reticulum, vacuoles, dense bodies and some mitochondria and scattered RNA particles $\times 11\,000$

they are rather frequently seen along with other non-fenestrated capillaries and arterioles. They have a distinct feature of overall irregular thickness of the endothelium and are marked by the presence of tall microvilli and polyp-like projections into the lumen (Fig 3A). The fenestrae are in the magnitude of 550 Å in diameter. Some of the pinocytotic vesicles on the basal surface retain the attenuated membrane (Fig 4B). Attenuated fenestrae are also seen on the luminal side of the endothelium covering the large vacuoles (Fig 4C). The endothelial cells do not contain filaments in bundle form. They are rich in RNA and some of the rough endoplasmic reticula are dilated. Vacuoles are frequently seen and pinocytotic vesicles are present in moderate number, more on the peripheral side even near the fenestra. The basal surface is completely covered by the continuous basement membrane which averages 1100 Å (range 990 to 1270 Å) when measured from the outer margin of the dense part to the endothelial cell. Pericytes make contact with the endothelial cell but do not extend over the fenestra. The vessels are surrounded by a thin layer of fibrils and by the modiolar

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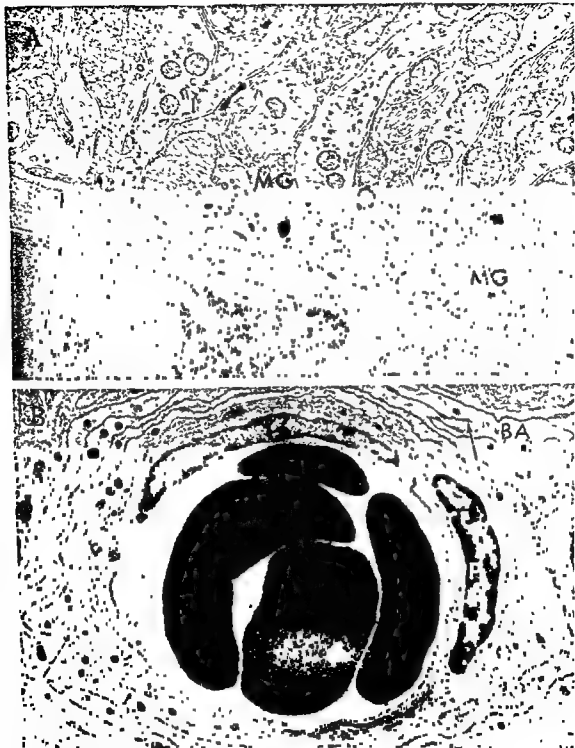


Fig 7 (A) A stria capillary abutted by marginal cells (MG) and intermediate cells (I) Pericyte (P) $\times 18\,500$ (B) The basal cells (BA) of the stria vasculans completely surround a capillary $8.3\ \mu$ Note

the lack of a perivascular fibrillar zone, and also numerous fascia occludentes which are indicated by dense patches between the cells (arrows) Pericyte (P) $\times 10\,500$

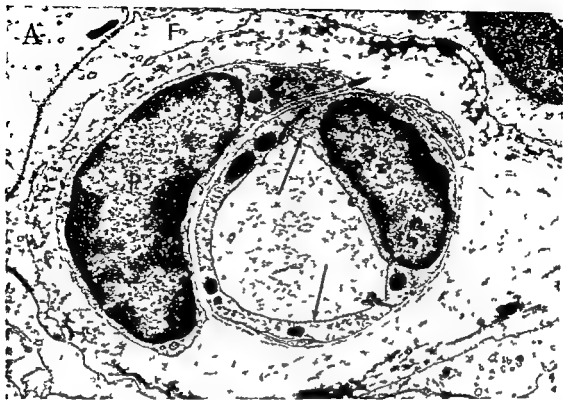


Fig. 8 (A) A capillary (4.2 μ) located in the spiral ligament adjacent to the scala vestibuli. The endothelium contains filaments in bundle form (arrows). The vessel is surrounded by a loose fibrous zone and fibrocytes (F). Pericyte (P) $\times 11,500$ (B) A capillary (5.5 μ) located in the spiral ligament adjacent to the scala tympani. Intracellular filaments in bundle form are lacking. Note a wide perivascular fluid space. Pericyte (P) $\times 15,000$.

located among the epithelial cells (marginal cells) of the labyrinth. The most obvious difference is their relation to adjacent cells, the vessels about the marginal cells frequently and the intermediate cells randomly (Fig 7 A). The vessels retain pericytes throughout, no distinct smooth muscle characteristics are found. Around the vessels are scant fibrils and, in a few instances, collagen fibers are demonstrable. The basement membrane is continuous and may branch, but its thickness varies considerably (740 to 1030 Å) with single or multiple layers. Only adjacent to or at the vessels do the marginal cells retain the basement membrane which becomes continuous or fused together with that of the endothelial cells. As the vessels enter or leave the stria vascularis, they are always tightly surrounded by the basal cells, which show numerous fasciae occludentes among themselves (Fig 7 B). The only intercellular pathway to and from the spiral ligament appears to be through the basement membrane of the vessels.

Stria vessels vary considerably in size, the average diameter is 6.4μ with a range of 3.5 to 12.7μ . The lumen generally retains a circular form, indicating very little contraction of the wall. The endothelial cell (height 0.1 to 0.5μ) is not fenestrated, and often shows marginal folds and some microvilli. The tight junction may be seen near the luminal surface or in the mid portion of the junction or may be observed in three or four different segmental zones or may be continuous throughout. The intracellular filamentous bundles seen in the arteriolar vessels are rarely demonstrable. The pinocytotic vesicles are found throughout the cytoplasm of the endothelial cells, though they tend to be more abundant at the basal portion. Pericytes show numerous short processes, and the filaments within the cytoplasm are located on the side close to the endothelial cell along the cell curvature, while pinocytotic vesicles are located more away from the lumen. The pericytes form a tight junction with the endo-

thelial cell by extending thin processes toward it, sometimes invaginating into it, also the main, broad part of the cell body extends and forms the tight junction. The pericytes are completely surrounded by the basement membrane except at the endothelial cell junction and also sometimes near the marginal and intermediate cells. The number of pericyte processes and endothelial cells in cross section of 57 stria vessels shows the endothelial cells number 42 and pericyte processes 63, which indicates that there are 1.5 times more pericyte processes. Pericyte processes are always demonstrable no matter how small the vessel is, even in a capillary without endothelial cell junctions, which is seen in the squirrel monkey.

The spiral ligament vessels seen behind the stria vascularis are generally small in diameter, averaging 5.8μ with a range of 4.5 to 15μ . The cell organelles are not much different from those seen in the stria vascularis, although a fair number of the vessels, particularly arterio-venous arcades, contain intracellular filaments. The basement membrane is thin, but the thickness varies considerably. The vessels located deeper in the spiral ligament are surrounded by scant fibrils and fibrocytes, but toward the stria vascularis they are more tightly surrounded by fibrocytes. Collagen fibers are not recognized. The average number of endothelial cells in cross-section of 34 vessels is 43 and that of the pericyte processes is 64, which is very close to the figures obtained in the stria vascularis. At the bifurcation point of capillaries in both the spiral ligament and the stria vascularis, pericyte processes are usually recognized but their structural details are not much different from those seen in the straight part of the vessels.

The vessels of the spiral prominence (7.0μ) do not differ from those seen in the spiral ligament behind the stria vascularis. Pinocytotic vesicles are more common at the basal surface. The basement membrane is generally thinner than that of the stria vascularis.

The vessels are surrounded by a rather thick layer of fibrils which in turn are enclosed by tightly packed fibrocytes. These spiral ligament cells (fibrocytes) are different from those seen in the deeper part of the spiral ligament, e.g. the mitochondria are often large and elongated and contain longitudinal cristae.

Capillaries come close to the external sulcus cells twisting around the cell processes in the lower cochlear turns. The vascular wall is usually interposed from the external sulcus cells by a thin layer of fibrils, though in some areas their basement membranes become continuous. The perivascular fibrocytes are similar to those seen in the spiral prominence and to those of the Rhesus monkey (Takahashi & Kimura, 1970). Their morphology differs from the modiolar cells which are described earlier.

The capillaries of the spiral ligament in the area adjacent to the scala tympani lack intracellular filaments in bundle form (Fig. 8B). However, in a few instances they are seen in larger venules. The cell organelles found in the endothelial cells of this area are no different from those found in the endothelial cells of the scala vestibuli. There is an indication that the number of endothelial cells in venules is smaller than that of arterioles of equal size (11 to 15 μ). In the arteriole the profile of the primitive smooth muscle cells or pericytes is larger and often longer. The number of endothelial cells and pericyte processes decreases from arterioles to capillaries and then increases in the venules. The distribution pattern of pinocytotic vesicles in arterioles and venules are similar, they are located more at the basal surfaces. The endothelial cell (cell height 0.2 μ) of the venule tends to be thinner than that of the arteriole, the luminal surface shows less irregularity in the venule while in the arteriole the surface tends to bulge and the nuclei are often crenated. There are no essential differences in the presence of microvilli and marginal folds in these two sides. The basement membrane of the arteriole is thicker (600 to 1344 Å)

than that of the venule (477 to 625 Å). On the scala tympani side the vessels are surrounded by a wide zone of fluid and by scant fibrils and fibrocytes (Figs. 8B, 11A) while on the arteriolar side there are more of these connective tissue elements.

The blood vessels of the osseous spiral lamina are shown in two different zones: one group within the bony channel and the other among the cochlear nerve fibers. The vessels within the bony canals are small and lack nerve fibers in the perivascular zone. The vessels among the cochlear nerve fibers are also small and their endothelial cells are surrounded by pericytes, fibrils and some collagen fibers. Unmyelinated nerve fibers are occasionally seen adjacent to these vessels, however, similar nerve fibers are seen among the cochlear nerve fibers. Some of the unmyelinated nerve fibers are very small while others are large, both are randomly located. The vessels of the limbus spiralis are similar to those of the spiral prominence and the external sulcus cells including the perivascular cells containing long mitochondria with longitudinally arranged cristae (Fig. 9). At the tympanic lip unmyelinated nerve fibers are sometimes seen adjacent to the vessels, their vesicles generally lack the central core (Fig. 10A).

Blood vessels of the basilar membrane (vas spiralis) are small with an average diameter of 4.9 μ ranging from 3.2 to 7.0 μ (Fig. 10B). The outstanding feature of this blood vessel is its location, which is close to the organ of Corti. There is nothing strikingly different about this vessel when compared with others. In the endothelial cell intracellular filaments in bundle form are sometimes seen. The vesicles are no more numerous than in other places and tend to occur more near the basal surface. The endothelial cells are not fenestrated and are completely surrounded by the basement membrane. Pericytes show numerous short processes and sometimes lack the basement membrane on the side away from the lumen. The vas spiralis is usually surrounded by a thick layer of homogeneous



Fig 9 A capillary (54 μ) in the limbus spiralis. Note a large fibrous area and perivascular fibrocytes containing long mitochondria with longitudinal cristae.

similar to those seen in the spiral prominence and external sulcus vessels. Pericyte (P) $\times 10,200$.

substance. This substance is finely granular, though it looks like short filaments, it also forms irregular aggregates which appear as dark patches. Outside the homogeneous layer are mesothelial cells partly or completely surrounding it and providing the appearance of perivascular channels (Hawkins, 1967). However, sometimes the mesothelial cells surround the vascular wall without an intervening layer of ground substance. Nerve fibers are not recognized.

The posterior spiral vein (Fig 11 B) and inferior cochlear vein (Figs 12 A, B) show similar morphological characteristics. The endothelium is thin (0.3 to 0.8 μ). The diameter of the spiral vein is 79 μ at the basal turn, and that of the inferior cochlear vein near the scala tympani is slightly larger (average 92 μ) than near the cranial opening (average 83 μ), the significance of which is not clear at present.

In the endothelial cells coated vesicles are many, and pinocytotic vesicles are located more often on the peripheral side (Fig 12 A). Bundles of intracellular filaments are sometimes seen in the endothelial cells of the inferior cochlear vein (Fig 12 B). The luminal surfaces show occasional prominent marginal folds and villous projections, and the basal surfaces reveal some cytoplasmic extensions. The tight junction is often very short, but the cell junction may be wide throughout (125 A). The basement membrane is thin and continuous, though in some areas a discontinuity is noted. Pericytes or primitive smooth muscle cells (Rhodin, 1968) are widely spaced, and the processes appear to overlap in some areas, the basement membrane is often lacking toward the endothelial cells. More peripherally, there is a wide space with loosely organized fibrils and a small number of fi-

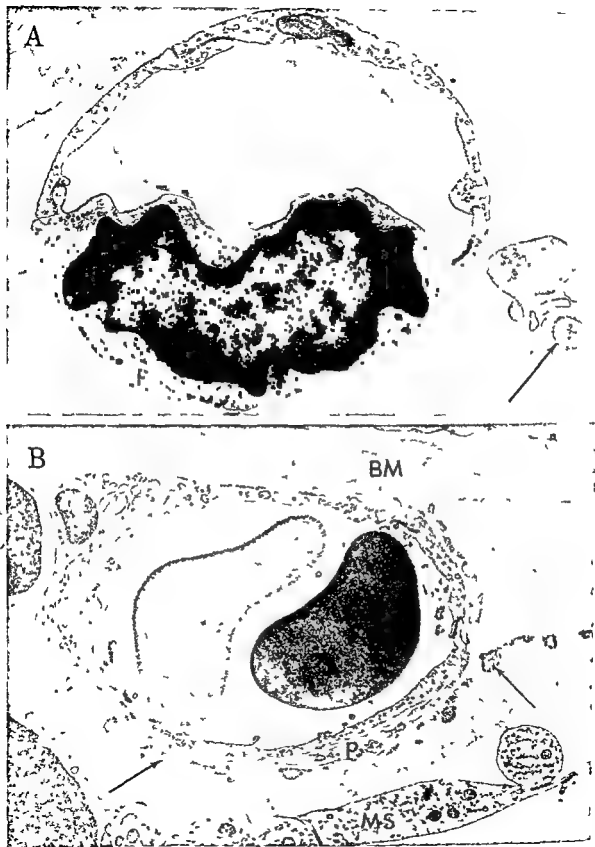


Fig 10 (A) A capillary ($53\ \mu$) at the tympanic lip of the limbus spiralis. Note some vesicles in the unmyelinated nerve fibers partly covered by the Schwann cell (arrow). Pericyte (P) $\times 21\ 300$. (B) The vas spiralis ($56\ \mu$) below the basilar membrane (BM). Note the homogeneous substance around the vessels dense patches (arrows) and mesothelial cells (MS). Pericyte (P) $\times 11\ 500$.



Fig. 11 (A) A collecting venule ($12\ \mu$ diameter) of the spiral ligament adjacent to the scala tympani. Note thin vascular wall and a wide fluid space on the outside. Compare with the arteriole in Fig. 6. Pericyte (P) $\times 10\ 000$. (B) Vascular wall of the pos-

terior spiral vein within the bony canal from the basal turn. The endothelium is thin, pericyte processes (arrows) are short and widely spaced, and the connective tissue is scant. $\times 14\ 000$.

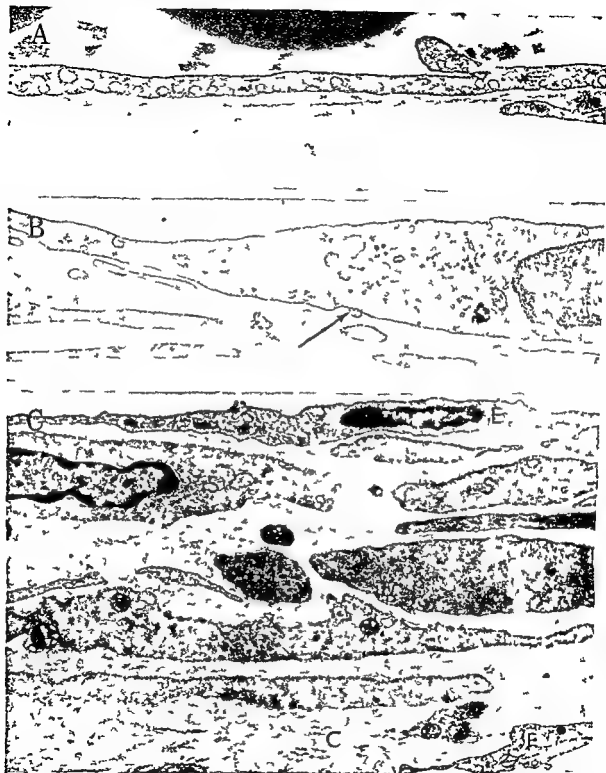


Fig 12 (A) The endothelium of the inferior cochlear vein with the bony canal. Note numerous pinocytotic vesicles at the basal surface and absence of pericytes $\times 34\,000$ (B) The endothelium of the inferior cochlear vein showing filaments in bundle form. Pericyte (arrow) $\times 17\,600$ (C) Vascular wall of the inferior petrosal sinus showing the endothelium (E), smooth muscle cells (S), fibrocytes (F) and collagen fibers (C) $\times 11\,000$

brocytes The inferior cochlear vein in the dura shows pericytes or primitive smooth muscle cells still widely spaced, but numerous collagen fibers and a few unmyelinated nerve fibers begin to appear Smooth muscle cells, in layers varying from one to six, are shown in the inferior petrosal sinus (Fig 12 C) They make contact with the endothelial cells with which filaments are scarce, and are not arranged in bundle form

DISCUSSION

Clear morphological differences are noted between the small blood vessels of the modiolus and those of the lateral wall of the membranous labyrinth The smooth muscle cells of the modiolar arterioles are similar to those of the peripheral vascular bed (Rhodin, 1967), but those of the radiating arterioles of the spiral ligament are flat, widely spaced and resemble pericytes The lack of a nerve supply and the lack of typical smooth muscle characteristics suggest that the ability of these vessels to contract is rather limited These vessels do not contract when the cervical sympathetic nerve is stimulated nor if adrenalin chloride is locally or systemically applied to these vessels (Perlman & Kimura, 1955) The major control of blood flow appears to be accomplished at the modiolus

The fenestrated vessels seen in the modiolar connective tissue are similar to those described earlier in the endolymphatic sac (Lundquist et al, 1964) The fenestrated vessels are presumed to be related to rapid transfer of fluid and solutes, and are reviewed extensively by Majno (1965) The fenestrated vessels seen in the modiolus are probably a branch of the main cochlear artery and are not expected to reach the membranous labyrinth The cells surrounding these fenestrated vessels show an unusually high protein metabolism, they are compared to the choroid plexus in producing cerebrospinal fluid, and are named the cochlear plexus by Balogh & Koburg (1965) The

present study shows that the modiolar cells (cells of the cochlear plexus) are essentially fibrocytes, while the cells of the choroid plexus are epithelial cells with a distinct basement membrane The modiolar cells may serve not only to support the vessels but also to participate in production or modification of modiolar fluid A brief ultrastructural description of the modiolar cells is provided earlier by Mootz & Musebeck (1970) It is well known that the cochlear vessels coil or twist within the connective tissue and also in the modiolar bone (glomeruli of Schwalbe, Hawkins, 1967) before becoming a straight line at the level of the radiating arterioles (Smith, 1951) At the coiled portion of the vessels, energy is presumably dissipated in reducing the pulsatile movement of blood The fenestrated vessels, which are apparently branches of these coiled vessels, may provide rapid transfer of fluid and may even play a role in reducing blood pressure These vessels are undoubtedly the major source of fluid surrounding the neural elements of the modiolus

Along the course of the cochlear blood vessels some differences in the cytoarchitecture of the endothelial cells are noted One variance is intracellular filaments in bundle form These filaments are more prevalent in the vessels in which blood flow is very fast Their arrangement may aid in propagating the movement of blood in the longitudinal direction by stretching and relaxing These endothelial filaments are common in other vessels (Bensch et al, 1964, Giacomelli et al, 1970), and have been considered to play a role in supportive (Cecio 1967) as well as contractile function (Fawcett, 1959) Contraction of the endothelial cell is known to occur even without pericyte contraction when histamine is applied (Majno et al, 1969)

Pinocytotic vesicles are common throughout the vascular channels Arterioles and venules show no essential difference in vesicle distribution Both of them show more vesicles at the basal surface Their presence is apparently not associated with the gas ex-

change of blood corpuscles, but appears to be closely associated with the cells involving active fluid transfer. They may serve to maintain fluid balance between blood and the interstitial space. The vesicles are generally considered vehicles of fluid or solute transfer across the cell, and/or they may carry some substance for intracellular use (Palade, 1953; Fawcett, 1966). Duvall et al (1971) demonstrated the passage of horseradish peroxidase through the stria endothelium by pinocytotic vesicles, not through the endothelium of the perilymphatic vessels. This is interpreted by Wersall et al (1973) to be due to the high density of vesicles in the stria capillaries.

The basement membrane is thicker on the arteriole side in comparison to the venule. Also it is often thick in the stria vascularis and in the fenestrated capillaries of the modiolus. Aging is apparently one of the factors involved in the increase in thickness (Gersh & Catchpole, 1949; Kurtz & Feldman, 1962). A very thick basement membrane is reported earlier in the stria vascularis of old humans (Kimura & Schuknecht, 1970). A correlation between blood pressure and the thickness of the basement membrane has been reported by Pease (1960). The thickest basement membrane is found in the renal glomerulus and in the renal capillaries where the capillaries are fenestrated and the blood pressure is high (Majno, 1965). The marginal cells of the stria vascularis are often compared to renal epithelium in their possible function of active fluid transport (Smith, 1957). However, the blood pressure within the stria capillaries is not known. The basement membrane is considered to be a product of the endothelial cells and to act as a selective filter, mechanical support for the endothelial cells, an anchorage for connective tissue, and a guide for regenerating vessels (Majno, 1965).

It is of interest to note that the ratio of pericyte processes to endothelial cells is just about the same in both the stria and the spiral ligament. Clear evidence of pericyte contraction has been lacking or the magnitude

of contraction is too difficult to determine under the light microscope. Under physiologic conditions neither mechanical nor drug stimulus is capable of inducing a visible change in the caliber of the vessels on the lateral membranous wall (Perlman & Kimura, 1955; Perlman et al, 1963). However, since these cells contain filaments similar to those seen in smooth muscle, they are considered capable of contraction (Rhodin, 1968). If they contract in the cochlea, the effectiveness must be greater in the vessels located adjacent to the scala vestibuli, and very little in the stria vascularis and the scala tympani side.

Except in the stria vascularis, adventitial cells are all fibrocytes or modifications of fibrocytes. These perivascular cells differ in morphology in different locations and are given specific names to identify them. The intercellular substances also differ, though their differences cannot be attributed to any specific cytoplasmic substance. The most remarkable differences are a sudden disappearance of collagen fibers (427 Å) in the membranous part of the labyrinth, an enormous increase in the ground substance (amorphous material) at the basilar membrane and an almost complete lack of fibrils and collagen fibers in the stria vascularis. The close arrangement of the marginal cells to the capillaries is not found among the vestibular secretory cells (dark cells) which are the counter part of the stria marginal cells, both of these cells are presumed to play a major role in maintaining a high potassium level in the endolymph (Kimura, 1969). An increase in endolymph pressure, however, may affect the stria capillaries more adversely because of this anatomical arrangement and the tight delineation established by the basal cells. The stria atrophy seen in the endolymphatic hydrops may be partly accounted for by this mechanism (Kimura, 1967). The close relationship between marginal cells and capillaries undoubtedly facilitates a turnover of endolymph.

ZUSAMMENFASSUNG

Die Ultrastruktur der cochlearen Blutgefäße wurde von den Arterien bis zu den Venen studiert. Einige wichtige Unterschiede zwischen den kleineren Gefäßen des Modiolus und den Gefäßen des membranösen Labyrinths wurden gefunden. Morphologische Befunde sprechen dafür, dass die Blutstromhauptkontrolle für das Cortische Organ im Modiolus bei Kapillaren mit Fenestern zwischen den Modioluszellen, die ungewöhnlich reich an Zellorganellen sind, gefunden. Diese Gefäße bilden wahrscheinlich die Hauptquelle der Modiolusflüssigkeit und könnten dazu dienen, den Blutdruck und die pulsierenden Bewegungen der Blutgefäße zu reduzieren. Intrazelluläre Filamente, die in Bündeln gruppiert sind, wurden oft in den endothelialen Zellen der Arterien, Arteriolen und arteriovenösen Arkaden gefunden. Sie sind meistens in Gefäßen mit sehr schnellem Blutstrom vorhanden. Die Kapillaren standen in Kontakt mit marginalen, intermedialen und basalen Zellen. Um die Venülen und die Venae cochlearis inferior fanden sich weit auseinander liegende Perizyten und seltene Fibrozyten. Es waren keine Nervenfasern in der direkten Umgebung der Gefäße des membranösen Labyrinths vorhanden, mit Ausnahme der tympanischen Lippe des Limbus spiralis.

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STAINING OF HAIR CELL NUCLEI BY A FLUORESCENT DERIVATIVE OF HEMICHOLINIUM-3

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Abstract The uptake of terphenyl HC-3, a fluorescent derivative of hemicholinium 3, by cochlear hair cells was investigated in normal and damaged guinea pig cochleas. The uptake of terphenyl HC 3 by hair cell nuclei is markedly reduced in Kanamycin and noise-induced cochlear damage. This reduction of terphenyl HC 3 uptake occurs before the appearance of the classic distortions in the gross morphology of the hair cells resulting from such damage.

Hemicholinium-3 (HC-3) is an agent which competitively inhibits cholinergic neurons from taking up choline and which thereby causes a reduction in the ability of such neurons to synthesize acetylcholine (ACh) (Schueler, 1960). This action has been of use in determining whether a given set of neurons are cholinergic or not. For instance, application of HC-3 has been shown to cause a steady diminution in the inhibitory influence of the olivo-cochlear bundle (OCB) thus suggesting that the OCB is cholinergic (Guth & Amaro, 1969).

Many derivatives of HC-3 have been synthesized in hope of delineating its active moieties and improving its actions. Among these derivatives is terphenyl-HC 3 (THC-3) a fluorescent compound. Haarstad (1973) first thought to make use of THC-3's fluorescent property as a histochemical agent with some specificity for cholinergic endings, the sites where choline tends to be most avidly taken up for the synthesis of ACh.

In the present work, THC-3 was applied to the organ of Corti in the hope that it would associate primarily with the only structures in the cochlea known to be cholinergic, the olivo-cochlear bundle (OCB) endings.

METHODS

Guinea pigs of both sexes weighing between 200 and 500 grams were used. Fluorescent staining of the organ of Corti was obtained in the following manner. After sacrificing the animals with overdoses of pentobarbital i.p., their temporal bones were removed, bullae opened and cochleae exposed. The "cap" of the cochlea and a portion of the base near the round window were cracked open and 2% O_2O_4 was allowed to flow through the cochlea. The cochleae remained submerged in the O_2O_4 for at least 2 min before being rinsed in three changes of physiological saline solution. THC-3 solution containing, THC-3 5×10^{-4} M and choline chloride 5×10^{-2} M (the choline was included to reduce background fluorescence, according to Haarstad, 1973), made just before use and continually warmed to keep the THC-3 in solution was then forced through the basal and apical ends of the cochlea. Cochleae were then submerged in the THC-3 solution for 10 min before being rinsed twice in physiological saline solution. Dissection and mounting of the basilar membrane was performed in 50% glycerol. These surface preparations were examined immediately by means of ultraviolet and phase-contrast microscopy. If not examined immedi-

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ately the fluorescence tended to "fade" in time. This fading is not seen in permanently-mounted sections from the central nervous system, so it is surmised that the THC-3 gradually diffuses out into the glycerol in which the basilar membrane pieces are mounted. The ultraviolet examination was made using a Leitz Ortholux UV microscope with a BG12 5 mm filter and a K510 suppression filter in dark field.

Three guinea pigs were treated with Kanamycin sulfate to induce hair cell damage. One received 400 mg/kg i.p. for 8 days and then was allowed 7 drug-free days before sacrifice. The other two guinea pigs also received 400 mg/kg i.p. for 8 days and no drugs for a week, but because both animals still had a vigorous Preyer reflex, drug treatment was reinstituted. One of these two animals received 400 mg/kg i.p. for a further 6 days, followed by 30 drug-free days before sacrifice. The third animal received 400 mg/kg for 8 days then no drug for 7 days, then 400 mg/kg for 6 days, then 600 mg/kg for 3 days and no drug for a final 14 days. All cochleae of these animals were treated as described above. Control animals were sacrificed along with the Kanamycin-treated animals.

Noise trauma was employed in 9 guinea pigs. These animals were all anaesthetized with pentobarbital (30 mg/kg i.p.). The middle ear on one side (control side) was then destroyed and the animals placed in a box for varying periods of time alongside a firebell which produced noise at 115 dB SPL. These animals were either kept for several days or sacrificed immediately and their cochleae treated as described above.

RESULTS

The arresting pattern of fluorescent dots clearly suggests association with the hair cells (Fig. 1). At first, this seemed to confirm the hypothesis that THC-3 was being taken up by the cholinergic OCB endings. That first impression was further strengthened by the observation that the intensity and distribution of fluorescence tended to parallel the distribution of OCB endings in the cochlea. That is, the outer rows of hair cells

in the basal end of the cochlea stained most intensely whereas in the middle and apical portions of the basilar membrane both inner and outer hair cells fluoresced but less intensely than at the basal end.

However, when the size and shape of the fluorescent dots was examined more closely it seemed clear that they were more likely associated with hair cell nuclei. The fluorescent dots are almost perfectly round and have a diameter nearly the same as outer hair cell nuclei. Furthermore, when the distances between nuclei within rows and between rows is measured they coincide almost exactly with these same distances measured between fluorescent dots.

To test the possible association of the "dots" with the hair cells it was decided to cause hair cell destruction by treating guinea pigs with ototoxic doses of Kanamycin. In all 3 treated animals no orderly rows of fluorescent "dots" appeared. This was so in spite of the fact that many hair cells and their nuclei appeared to be intact (Figs. 2 & 3) although much hair cell destruction and scarring was seen. This unexpected result, i.e. the lack of THC-3 uptake by apparently intact hair cell nuclei, was set aside until the results of another experimental stratagem could be studied. It was decided to attempt to disrupt the cochlear hair cells by using high intensity noise prior to application of THC-3. Once again, although most of the hair cells and their nuclei appeared to be intact when viewed under phase-contrast microscopy (Fig. 4), either no orderly rows of fluorescent dots were seen or there was a marked difference in fluorescent intensity between the control (middle ear destroyed prior to noise trauma) and treated cochleae. That THC-3 still gains access to the organ of Corti is witnessed by the fact that other cochlear structures presumably supporting cell nuclei which appear elliptical rather than round and not in orderly rows, continue to take it up and fluoresce (Fig. 5). The conclusion drawn from these results is that the ability of the hair cells to take up THC-3 is very sensitive to Kanamycin and noise induced cochlear damage. In fact, the weakening or actual loss of fluorescent staining

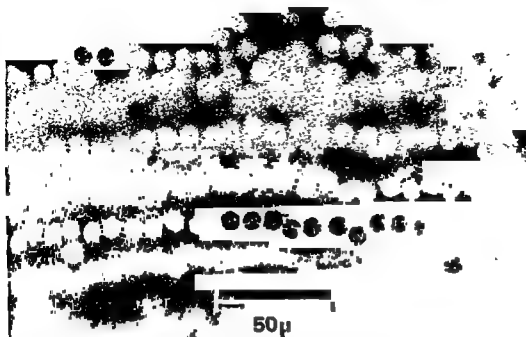


Fig. 1. Ultraviolet light photomicrograph of guinea pig basilar membrane, the basal turn, showing the regular rows of round fluorescent dots produced by exposing osmicated basilar membrane to THC-3

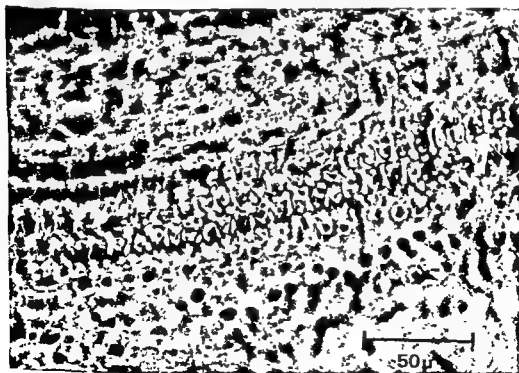


Fig. 2. White light phase contrast photomicrograph of hair cells from the middle turn of Kanamycin-treated guinea pigs

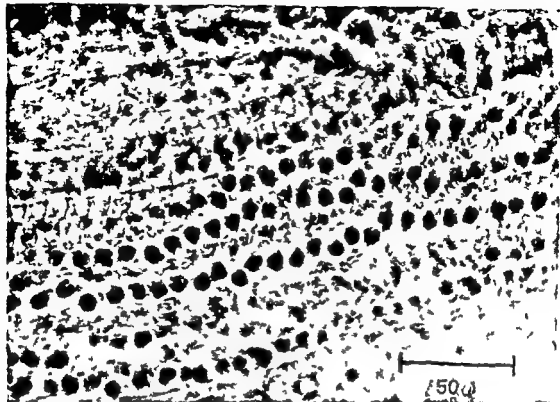


Fig. 3 White light phase contrast photomicrograph of hair cell nuclei from the same region as Fig. 2.

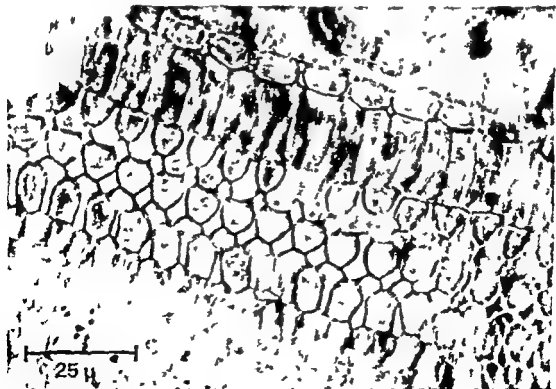


Fig. 4 White light phase contrast photomicrograph of hair cells from middle turn of the cochlea. The guinea pig in this case was subjected to 115 dB S.P.L. bell tone for 60 min. Middle ear intact.

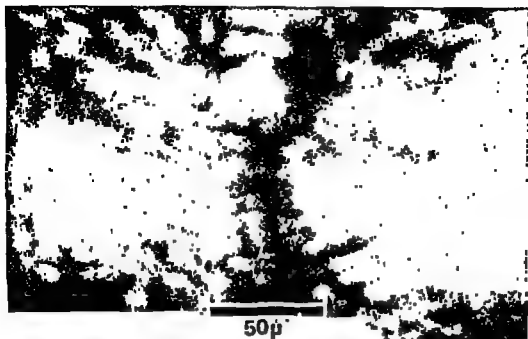


Fig 5 Ultraviolet light photomicrograph of a similar region as Fig 4 Middle ear in cat

in response to noise or Kanamycin occurs before the now-classic gross distortions of cochlear morphology such as hair cell nuclear swelling, and breakdown, and actual scarring of the hair cells. The main purpose of this paper is, in fact, to apprise otologic researchers of this fact and of its possible use as a tool in further otologic research.

The particular cochlear distribution of the fluorescence is of interest in that it appears to parallel the well-known pattern of damage induced by noise or ototoxic agents as well as the olivo-cochlear bundle endings.

The authors propose next to study whether the uptake of THC-3 by hair cell nuclei is related to the uptake of choline by these structures.

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ZUSAMMENFASSUNG

Die Aufnahme von Terphenyl HC 3, einem fluoreszierenden Derivat von Hemicholinium-3, durch die Haarzellen im Cortischen Organ des Meerschweinchens wurde untersucht, und zwar sowohl in normalen als auch in

geschädigten Cochleae. Die Aufnahme von Terphenyl-HC-3 durch den Zellkern ist nach Beschädigung der Cochlea durch Lärm oder Kanamycin bedeutend reduziert. Diese Reduktion tritt vor dem Auftreten der klassischen Verzerrung in der Morphologie der Haarzelle ein, welche durch solche Beschädigung verursacht wird.

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Note added in proof: Since submission of this manuscript a paper has appeared (Goldstein, A. J. 1973. Permeability of the organ of Corti. *Ann. Otol.* 82, 166) in which a similar lack of staining of cochlear hair cells following sound stimulation was reported. The fluorescent stain employed was fluorescein diacetate. The dissimilarity of the two stains employed suggests that the change induced by sound affects some general process such as permeability as Goldstein suggests.

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ZUR REIZINTERVALL-ABHÄNGIGKEIT AKUSTISCH
EVOZierter POTENTIALE

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(Eingegangen am 29 Juli, 1973)

Abstrakt Die Abhängigkeit der N_1 - P_2 -Amplitude und der Latenzen akustisch evozierter Potentiale vom Reizintervall (0,2 s bis 10 s) wurde bei den Reizstärken 20, 30, 50, 70 und 90 dB u S gemessen. Im untersuchten Intervallbereich erreichen die Amplituden (außer der 20-dB-Kurve) noch nicht ihren Höchstwert. Jedoch zeigt eine Extrapolation der Kurven über 10 s hinaus, daß bereits bei 10 s mindestens 90 % der Maximal-Amplitude erreicht werden. Die prozentuale Amplitudenabnahme bei kürzeren Intervallen (bezogen auf den Wert bei 10 s) ist für alle Reizstärken etwa gleich. Durch Umzeichnen der Amplituden-Meßwerte wird eine Reizstärke — Erregungskennlinie mit der Intervalldauer als Parameter erhalten. Mit der Intervalldauer steigen die N_1 - und P_2 -Latenzen signifikant an, $t_{L_{P_2}}$ stärker als $t_{L_{N_1}}$. Bei kurzen Intervallen durchlaufen beide Kurvenscharen ein Minimum. Eine Änderung des Schallpegels bewirkt im wesentlichen nur eine Parallelverschiebung der Kurven, deren Ausmaß eine Funktion des Schallpegels ist. Infolge dieses Verhaltens ist die Latenzdifferenz der Antworten bei kurzen und bei langen Intervallen nahezu unabhängig von der Reizstärke.

Die Tatsache, daß die N_1 - P_2 -Amplitude des akustisch evozierten Potentials (AEP) stark von der Reizintervall-Dauer abhängt, ist allgemein bekannt. Der funktionelle Zusammenhang zwischen Intervalldauer und AEP-Amplitude sowie der Einfluß der Intervalldauer auf die Form der Kennlinie (AEP-Amplitude $\sim f(L_{\text{Aud}}/\text{dB u S})$)¹ werden jedoch nur in wenigen Arbeiten angegeben und erstrecken sich nicht über den gesamten interessierenden Intensitäts- bzw. Intervallbereich. Davis et al. (1966) haben die Abhängigkeit der N_1 - P_2 -Amplitude des AEP

vom Reizintervall für Tonpips (gefilterte Clicks, 1200 Hz, 85 dB ISO) angegeben. Nelson & Lassman (1968) haben den Zusammenhang zwischen Stimulus-Intervall und AEP-Amplitude mit 20 ms Tonbursts bei einer Reizstärke (60 dB) für mehrere Frequenzen gemessen. Beide Ergebnisse sind wegen der Unterschiede in der Reizart nicht direkt vergleichbar. Gjerdingen & Tomsic (1970) haben die Recovery-Funktionen der Potentiale, die durch Tone, elektrische Schocks, Vibration und Lichtreize evoziert wurden, verglichen.

Ziel unserer Arbeit zu dieser Fragestellung war es, die Abhängigkeit der AEP-Amplituden und -Latenzen von der Reizintervalldauer bei einer Frequenz (1000 Hz) über den gesamten interessierenden Schallpegel-Bereich zu untersuchen.

METHODIK

Die Untersuchungen wurden in einer schallisolierten Kammer durchgeführt. Als Versuchspersonen (VP) dienten 10 normalhörende Studenten im Alter von 20–25 Jahren. Die Elektrodenlage war Vertex gegen Mastoid, Erdung erfolgte am Ohrfläppchen. Als Reizfrequenz wurden 1000 Hz gewählt, die Reizdauer war 500 ms mit 25 ms Anstiegs- und Abfallzeit (Die Anstiegszeit ist in den 500 ms enthalten). Das verwendete Audiometer der Firma PAR ist nach ISO-Norm kalibriert. Die Mittelung wurde mit einem Averager vom Typ DL 102A der Firma DATALAB durchgeführt, dessen

¹ $L_{\text{Aud}} \sim 20 \log(p_{\text{Kuppler}}/p_0) - L_0$ dB über Schwelle
 p_{Kuppler} = Schalldruck im NBS 9A-Kuppler
 $p_0 = 2 \cdot 10^{-4}$ N/m²
 L_0 ISO = Schwellen-Kupplerschallpegel nach ISO

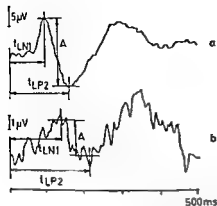


Abb 1 Demonstration der Methodik der Potentialausmessung (a) Potential mit geringem Störsignalanteil (gemittelt bei 70 dB u S und 2 s ISI) (b) Potential mit hohem Störsignalanteil (gemittelt bei 20 dB u S und 1 s ISI) Anzahl der Mittelungsschritte in beiden Fällen $n=64$

besonderer Vorteil die Möglichkeit einer automatischen Artefact-Unterdrückung ist. Als EEG-Verstärker wurde der Verstärker Typ 113 der Firma PAR mit einem Eingangswiderstand von 100 M Ω verwendet. Die obere Grenzfrequenz war 30 Hz, die untere 0,3 Hz.

Während der Untersuchung lasen die VP Texte nach eigener Wahl. Als Amplitude des AEP wird die Differenz zwischen N_1 und P_2 bezeichnet. Da bei geringen Reizstärken und kurzen Reizintervallen das Signal-Stör-Ver-

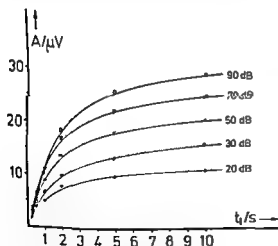


Abb 2 Abhängigkeit der N_1, P_2 -Amplitude des AEP von der Pausendauer zwischen den Reizen mit dem Schallpegel als Parameter (Standardabweichungen siehe Abb 3)

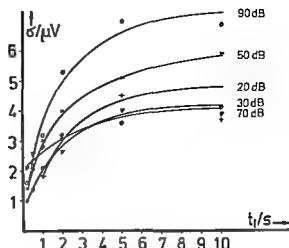


Abb 3 Standardabweichung der AEP Amplituden in Abhängigkeit von der Pausendauer zwischen den Reizen

hältnis ungünstiger ist, ergibt sich als Mittelungsergebnis bei den gewählten 64 Mittelungsschritten n U kein glatter Kurvenzug. Die dabei angewandte Methodik der Ausmessung veranschaulicht Abb 1.

Bei jeder VP wurde die Intervall-Abhängigkeit bei den Audiometer-Reizstärken 20, 30 und 50 dB u S je 4mal, bei 70 und 90 dB u S je 3mal gemessen. Alle Meßpunkte jeder Kurve sind daher Mittelwerte von jeweils 40 bzw. 30 Einzelpotentialen (10 VP). Geprüft wurde bei den Interstimulus-Intervallen (ISI) 0,2, 0,5, 1, 2, 5, 10 s. Als ISI wird die Dauer der Pause zwischen zwei aufeinanderfolgenden Reizen bezeichnet.

ERGEBNISSE

Abb 2 zeigt die ermittelte Abhängigkeit der AEP-Amplitude von der Dauer der Pause zwischen den Reizen bei 90, 70, 50, 30 und 20 dB u S. Zur besseren Übersichtlichkeit wurde die für alle Meßpunkte berechnete Standardabweichung σ^1 gesondert in Abb 3 dargestellt.

Aus Abb 2 ist ersichtlich, daß auch bei einem ISI von 10 s die bei dem jeweiligen Schallpegel

¹ Obwohl es sich hier um die Standardabweichung in der Stichprobe und nicht in der Grundgesamtheit handelt, wurde statt s die Bezeichnung σ gewählt, um eine Verwechslung mit der Maßeinheit der Intervalldauer (Sekunde) zu vermeiden.

ZUR REIZINTERVALL-ABHÄNGIGKEIT AKUSTISCH
EVOZierter POTENTIALE

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(Eingegangen am 29 Juli, 1973)

Abstrakt Die Abhängigkeit der N_1 - P_2 -Amplitude und der Latenzen akustisch evozierter Potentiale vom Reizintervall (0,2 s–10 s) wurde bei den Reizstärken 20, 30, 50, 70 und 90 dB u S gemessen. Im untersuchten Intervallbereich erreichen die Amplituden (außer der 20-dB-Kurve) noch nicht ihren Höchstwert. Jedoch zeigt eine Extrapolation der Kurven über 10 s hinaus, daß bereits bei 10 s mindestens 90 % der Maximal-Amplitude erreicht werden. Die prozentuale Amplitudenabnahme bei kürzeren Intervallen (bezogen auf den Wert bei 10 s) ist für alle Reizstärken etwa gleich. Durch Umzeichnen der Amplituden-Meßwerte wird eine Reizstärke – Erregungskennlinie mit der Intervalldauer als Parameter erhalten. Mit der Intervalldauer steigen die N_1 - und P_2 -Latenzen signifikant an, $t_{L_{P_2}}$ stärker als $t_{L_{N_1}}$. Bei kurzen Intervallen durchlaufen beide Kurvenscharen ein Minimum. Eine Änderung des Schallpegels bewirkt im wesentlichen nur eine Parallelverschiebung der Kurven, deren Ausmaß eine Funktion des Schallpegels ist. Infolge dieses Verhaltens ist die Latenzdifferenz der Antworten bei kurzen und bei langen Intervallen nahezu unabhängig von der Reizstärke.

Die Tatsache, daß die N_1 - P_2 -Amplitude des akustisch evozierten Potentials (AEP) stark von der Reizintervall-Dauer abhängt, ist allgemein bekannt. Der funktionelle Zusammenhang zwischen Intervalldauer und AEP-Amplitude sowie der Einfluß der Intervalldauer auf die Form der Kennlinie (AEP Amplitude = $f(L_{\text{Aud}}/\text{dB ü S})$)¹ werden jedoch nur in wenigen Arbeiten angegeben und erstrecken sich nicht über den gesamten interessierenden Intensitäts- bzw. Intervallbereich. Davis et al. (1966) haben die Abhängigkeit der N_1 - P_2 -Amplitude des AEP

vom Reizintervall für Tonpips (gefilterte Clicks, 1200 Hz, 85 dB ISO) angegeben. Nelson & Lassman (1968) haben den Zusammenhang zwischen Stimulus-Intervall und AEP-Amplitude mit 20 ms Tonbursts bei einer Reizstärke (60 dB) für mehrere Frequenzen gemessen. Beide Ergebnisse sind wegen der Unterschiede in der Reizart nicht direkt vergleichbar. Gjerdingen & Tomsc (1970) haben die Recovery-Funktionen der Potentiale, die durch Töne, elektrische Schocks, Vibration und Lichtreize evoziert wurden, verglichen.

Ziel unserer Arbeit zu dieser Fragestellung war es, die Abhängigkeit der AEP-Amplituden und -Latenzen von der Reizintervalldauer bei einer Frequenz (1000 Hz) über den gesamten interessierenden Schallpegel-Bereich zu untersuchen.

METHODIK

Die Untersuchungen wurden in einer schall isolierten Kammer durchgeführt. Als Versuchspersonen (VP) dienten 10 normalhörende Studenten im Alter von 20–25 Jahren. Die Elektrodenlage war Vertex gegen Mastoid, Erdung erfolgte am Ohrfläppchen. Als Reizfrequenz wurden 1000 Hz gewählt, die Reizdauer war 500 ms mit 25 ms Anstiegs- und Abfallzeit. (Die Anstiegszeit ist in den 500 ms enthalten.) Das verwendete Audiometer der Firma PAR ist nach ISO-Norm kalibriert. Die Mittelung wurde mit einem Averager vom Typ DL 102A der Firma DATALAB durchgeführt, dessen

¹ $L_{\text{Aud}} = 20 \log(p_{\text{Kuppler}}/p_0) - L_{0 \text{ ISO}}/\text{dB}$ über Schwelle
 p_{Kuppler} = Schalldruck im NBS 9A-Kuppler
 $p_0 = 2 \cdot 10^{-3} \text{ N/m}^2$
 $L_{0 \text{ ISO}}$ = Schwellen Kupplerschallpegel nach ISO

Fruhstorfer et al (1969), Rothman et al (1970) und Ruhm (1971) fanden ebenfalls, daß 10 s Intervalle bei akustischer Reizung ausreichend sind für vollständige Erholung.

Nelson & Lassman (1968) fanden einen linearen Zusammenhang zwischen der AEP-Amplitude und dem logarithmisch aufgetragenen Reiz Intervall im Bereich 0,25–6 s. Dieses Ergebnis wird durch die vorliegenden Messungen nicht bestätigt. Erklärt werden kann dieser Unterschied und auch die bedeutend kleineren Amplituden in der genannten Arbeit eventuell durch den wesentlich kürzeren Ton-Reiz (20 ms), den die Autoren verwendeten. Die prozentuale Amplitudenverminderung bei kürzeren Intervallen (bezogen auf den Wert bei 10 s) ist für alle gemessenen Reizstärken etwa gleich. Webster (1972) fand ebenfalls keine Differenzen zwischen der Amplitudenabnahme bei hohen und bei niedrigen Reizstärken. Da er auch keine Dis-habituation nachweisen konnte, schlußfolgert er, daß die Amplitudenminderung akustisch evozierter Potentiale bei kurzen Reiz-Intervallen im wesentlichen nicht durch Habituation bewirkt wird.

Davis et al (1966) konnten keine Latenzänderungen akustisch evozierter Potentiale in Abhängigkeit vom ISI nachweisen. Nelson & Lassman (1968) gaben nur für P_2 und N_2 eine signifikante Latenzverlängerung bei längeren Intervallen an. Die in der vorliegenden Arbeit dargestellte signifikante Änderung (0,5%-Niveau) auch der Latenz von N_1 wird durch Ergebnisse von Karnahl & Benning (1972) bestätigt. Die Autoren haben die Abhängigkeit der Latenz von der Reizstärke bei zwei unterschiedlichen Reiz-Intervallen gemessen (1,3 s–3,6 s Intervalle = „adaptierter Zustand“, 5 s–20 s Intervalle = „nichtadaptierter Zustand“). Die mittleren Intervalle der zufallsverteilten Reizfolgen sind leider nicht angegeben. Die Autoren fanden für den nichtadaptierten Zustand signifikant längere P_1 -, N_1 - und P_2 -Latenzen. Die Latenzdifferenz adaptiert–nichtadaptiert ist für P_2 am kleinsten und für alle 3 Wellen nahezu unabhängig vom Schallpegel.

Zeichnet man für die Parameter $t_i = 10$ s und

$t_i = 2$ s mit den zu diesen Intervallen gehörenden Meßwerten aus Abb. 4 die Verläufe $L_{N1} = f(L_{\text{Aud.}})$ und $L_{P2} = f(L_{\text{Aud.}})$, so erhält man qualitativ dieselben Ergebnisse. Ein quantitativer Vergleich ist nicht möglich wegen der fehlenden Angabe der mittleren Reiz-Intervalle in der genannten Arbeit.

Über das deutliche Minimum der N_1 - und P_2 -Latzen bei kurzen Intervallen in Abb. 4 wird von keinem der zitierten Autoren berichtet. Die Vermutung, daß das Ansteigen der Latenzen bei kurzen ISI artifizuell ist und eventuell durch eine Überlagerung der Reizantwort mit dem Off-Effekt der Antwort auf den jeweils vorhergehenden Reiz verursacht wird, ist nicht begründet. Nach Messungen von Johannsen et al (1972) sind die Latenzen des Off-Effektes verglichen mit dem On-Effekt kürzer, so daß auch bei 0,2 s Reizintervallen sich nur sehr späte Komponenten ($t_L > 300$ ms) der Off-Antwort mit dem N_1 - P_2 -Komplex der nächsten On-Antwort überlagern können. Da die Amplitude des Off-Effektes jedoch bei der verwendeten Reizart nur 10–20% der Amplitude der On-Antwort beträgt (Spreng, 1969), kann der Einfluß einer Überlagerung vernachlässigt werden.

Von Beagley & Kellogg (1970) wurden Reizstärke-Erregungskennlinien für Intervalle von 20 s und 1,25 s angegeben, die sehr gut mit den entsprechenden Kennlinien der Abb. 5 übereinstimmen, wenn berücksichtigt wird, daß eine Verlängerung des Intervalls von 10 s auf 20 s keinen bedeutenden Amplitudenzuwachs erzielen kann. Ein Vergleich mit den von Stange (1971) veröffentlichten Kennlinien für den adaptierten und den nichtadaptierten Zustand ist schwierig, da keine Angaben über die beiden mittleren Reiz-Intervalle gemacht werden. Auch wird in der genannten Arbeit der logarithmische Maßstab für die Amplituden bevorzugt, während für unsere Meßergebnisse die lineare Darstellung geeigneter erscheint.

Die physiologische Grenze der Fähigkeit zur Antwortsteigerung bei Erhöhung der Reizstärke ist eine Funktion des Reiz-Intervalls. Aus Abb. 5 geht hervor, daß bei 1 s Intervallen diese Grenze bei etwa 75 dB ü S erreicht ist. Bei

10 s Intervallen liegt sie sicher oberhalb 100 dB ü S. Wegen der Unzumutbarkeit für die Versuchspersonen wurden oberhalb 90 dB ü S keine Messungen durchgeführt.

Die Ergebnisse wurden im Rahmen der WK Herzkreislauf Erkrankungen im Auftrag des Ministeriums für Gesundheitswesen der DDR erarbeitet. Wir danken dem Leiter der Arbeitsgruppe MdT Herrn Oberarzt Dr. Michel sowie Herrn Dr. Ullrich für die Unterstützung.

SUMMARY

The dependence of the N_1 - P_2 amplitudes and latencies of auditory evoked potentials upon the interval between the stimuli (0 s up to 10 s) was measured at 20, 30, 50, 70, and 90 dB above the audiometer threshold (ISO). Within the investigated range of intervals, the curves (the 20 dB-curve excluded) do not reach their top value. Extrapolation of the curves beyond 10 s shows, that at least 90% of the maximum amplitude will be reached at 10 s. The percental decrease of the amplitudes at shorter intervals (related to the value at 10 s) will be approximately equal for all intensities. When altering the drawing of the measured amplitude values, a curve set of input-output functions (amplitude vs. sound level with the duration of intervals as parameter) will be obtained. With increasing duration of intervals, the N_1 - and P_2 -latencies increase significantly, i.e. t_{LP_2} more than t_{LN_1} . At short intervals, both latency curve sets run through a minimum. Alteration of the sound level causes parallel displacement of the curves only, the dimension of which is a function of the sound level. Due to this behaviour, the latency difference of the answers for long and short intervals is approximately independent of the stimulus intensity.

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LABYRINTHINE MORPHOLOGY AND TEMPERATURE IN CRYOSURGERY (GUINEA PIG)

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Abstract The effects of cryosurgery on the guinea pig labyrinth as visualized by light and electron microscopy are presented. The material studied was taken from animals in which the intralabyrinthine temperatures were known (Morrison & Lundquist 1973). Between +10 and +19°C changes first appear in the mitochondria located in the apical part of hair cells close to the endolymph. At cooler temperatures intracytoplasmic vacuoles appear, clumping of nuclear chromatin can be seen, and as the changes progress they involve the supporting cells, secretory areas and connective tissue as well. At even colder levels (+4° +10°C) the epithelium becomes fragmented and lifts away from the connective tissue. However, this debris is cleared away within 4 days and the crista is healed by a layer of flattened supporting cells.

Several surgical procedures have been developed in an attempt to relieve patients with Meniere's disease of their vertigo and tinnitus without causing further deterioration of hearing. A recent method is the cryosurgical alteration of vestibular function achieved by placing a cryosurgical probe on the lateral semicircular canal (Cutt et al, 1965, Wolfson & Cutt, 1971, Wolfson, 1973, Tabor et al, 1972). Little is known about what actually takes place within the labyrinth during cooling. In a recent publication Morrison & Lundquist (1973) presented the temperature changes that can be achieved in various parts

of the guinea pig labyrinth when the lateral semicircular canal ampulla is cooled by a cryosurgical probe, and the advance of the cooling front was shown for different probe tip temperature levels. The intralabyrinthine temperature recordings were correlated with morphological changes as seen by light microscopy. This investigation presents, in greater detail, the histological changes caused by a cryosurgical intervention in the labyrinth. The influence of survival time and the relationship to intralabyrinthine temperatures will be discussed as well.

MATERIALS AND METHODS

Materials for morphologic study were taken from 18 young guinea pigs in which the bone over the left lateral semicircular canal ampulla was exposed to a cryosurgical probe at two temperature levels, -50°C and -75°C, for various periods of time. The animals were further subdivided into groups having different survival times after the freezing procedure, so that specimens were taken either immediately after freezing utilizing an intravital labyrinthine perfusion of osmium, or else the animal was allowed to live for 2 hours, 1 day, or 4 days after cryosurgery. After removal of the temporal bones the intralabyrinthine structures were fixed in 1% cold osmium tetroxide, dissected, dehydrated, embedded in Epon, and cut on an LKB Ultratome.

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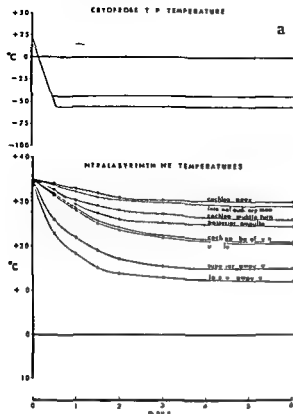


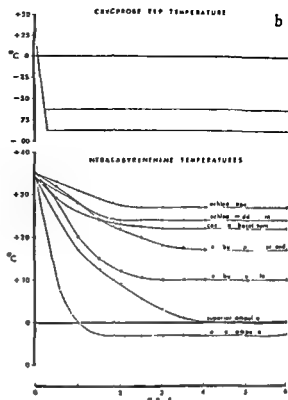
Fig 1 Temperature changes in the guinea pig labyrinth during the application of a cryoprobe* on the

For examination by light microscopy 1 μm thick sections were used, stained with toluidine blue. Thin sections mounted on copper grids were stained with uranyl acetate and lead citrate and examined with a Siemens Elmiskop I electron microscope.

The cristae from the lateral and superior semicircular canal ampullae as well as the macula of the utricle were examined in each case. Cochlear preparations, as well as preparations of the saccule and posterior ampulla, were made from several of the more severely cooled specimens.

RESULTS

The morphologic changes caused by cry-surgery on the vestibular labyrinth will be presented in two main sections. Firstly, we will examine the various changes seen im-



lateral ampulla maintained at -50°C (a) or -75°C (b) (Taken from Morrison & Lundquist 1973)

mediately after the cooling procedure and secondly, the effects of survival time after cryosurgery will be described.

Immediate morphological effects of cooling

When the guinea pig labyrinth is cooled by a cryosurgical probe a very wide range of morphological changes may be produced, depending on several factors, the most important being the degree of cooling. With the application of a -50 to -75°C cryoprobe on the lateral semicircular canal ampulla, intralabyrinthine temperatures from -3°C to $+30^{\circ}\text{C}$ are obtained (Fig. 1). The temperatures described are actual intralabyrinthine recordings.

Minimal changes

When the epithelial temperature is lowered to +18 or +19°C the morphological changes are very slight and can only be seen with the

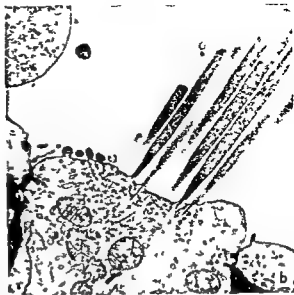


Fig 2 Light micrograph (a) and electron micrograph (b) of a lateral ampullary crista that had been subjected to very minimal cooling. By light microscopy

it is normal appearing however electron microscopy reveals early mitochondrial degeneration (a Toluidine blue $\times 200$ b $\times 10\,000$)

electron microscope. This is demonstrated by Fig 2 in which the light micrograph of an ampullary crista is normal appearing, but electron microscopy reveals some destruction of the internal cristae of the mitochondria, especially in the apical region of the hair cells

close to the endolymph. At slightly lower temperatures ($+10$ to $+15^{\circ}\text{C}$) vacuoles begin to appear in the cytoplasm, initially in the sub-apical and supranuclear regions but with still more cooling they are seen throughout the hair cell cytoplasm (Fig 3)

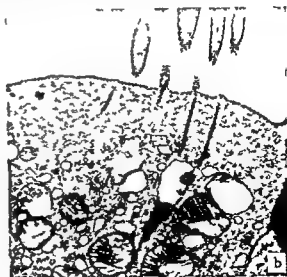


Fig 3 Guinea pig crista epithelium exposed to an intralabyrinthine temperature of about $+10$ – $+15^{\circ}\text{C}$. There is widespread damage to mitochondria and cyto-

plasmic vacuole formation. Fig 3b is a higher magnification of the apex of the type I hair cell shown in (a) (a $\times 2\,500$ b $\times 13\,000$)



Fig 4 Ballooning of surface membranes and extrusion of cytoplasm in type I hair cells mildly affected

by cryosurgery. The kinocilia are distorted. Note the mitochondrial changes as well ($\times 22\,500$)

Some ballooning of the hair cell surface membrane and cytoplasm into the endolymph, alongside the cuticular plate, is frequently seen in normal specimens and is attributed to fixation artifact. However, in the mildly affected cryosurgical specimen this is more widely spread, and an increase in cytoplasmic extrusions can be seen (Fig 4).

Slightly beyond the stage of these most subtle

changes some clumping of nuclear chromatin becomes visible, and at this stage of degeneration changes are usually visible in the light microscope.

Moderate changes

These occur at a temperature of approximately $+4$ to $+10^{\circ}\text{C}$. As mentioned above, the more extensive intracellular vacuolization together



Fig 5 Early cryosurgical changes as seen on light microscopy. There is noticeable edema between the epithelium and connective tissue in the transitional zone, and very early changes can just be detected in the sensory areas (Toluidine blue, $\times 235$)



Fig 6 (a) Large vacuoles and empty mitochondria are seen in the hair cell cytoplasm to the right. Further left is the nerve chalice containing distorted



mitochondria as well. The supporting cell (top left) is normal appearing ($\times 22\,000$). (b) Well developed myelin figures in a nerve chalice ($\times 33\,000$)



Fig. 4 Ballooning of surface membranes and extrusion of cytoplasm in type I hair cells mildly affected

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Fig 8 Light micrographs of two lateral cristae each exposed to a -75°C cryoprobe for 3 minutes (Intra labyrinthine temperature about -3°C) Note the



epithelial fragmentation nuclear pyknosis and cellular debris floating into the endolymphatic fluid (Toluidine blue $\times 200$)

specimens taken from regions of the vestibular system where the degree of cooling was greatest. Again however, the damage is found to be more extensive in the hair cells than in other elements.

The more severely damaged mitochondria are usually seen as distended, almost empty envelopes, but myelin figures may also be present, and are considered to be remnants of destroyed mitochondria (Fig 6). Mitochondrial changes in the nerve endings are less pronounced than those seen in the hair cells.

Prior to the most severe changes with breakdown of the epithelium, the intracellular vacuoles coalesce and become larger, and quite large vacuoles are now found in supporting cells as well (Fig 7).

Severe changes

Typical severe changes in the lateral semicircular canal ampulla as seen by light microscopy are shown in Fig 8. The sensory epithelium is generally disorganized and in many areas it has lifted away from the underlying connective tissue. However, even in the midst

of areas of marked destruction some continuity of the reticular lamina can be seen. Many of the nuclei are pyknotic. Cellular debris is found floating in the endolymphatic fluid. The epithelial disruption is consistently more pronounced on the canal side of the ampullary crista, which is the one closest to the site of cryoprobe application.

The central core of the crista is edematous and the blood vessels are congested with red blood cells. The dark cells of the secretory region appear, on light microscopy, to be irregular but have survived the cooling much more than the sensory areas above them.

The electron microscopic appearance of the epithelial disruption can be seen in Fig 9. All cells are fragmented and most of the nuclei are pyknotic. The apical region of a destroyed hair cell can be seen in which the general relationship between the reticular lamina and the cuticular plate is maintained, however the zones of contact between the cells, with the tight junctions, are lost. The arrangement between the sensory hairs and the cuticle is intact as are the hair rootlets within it, however

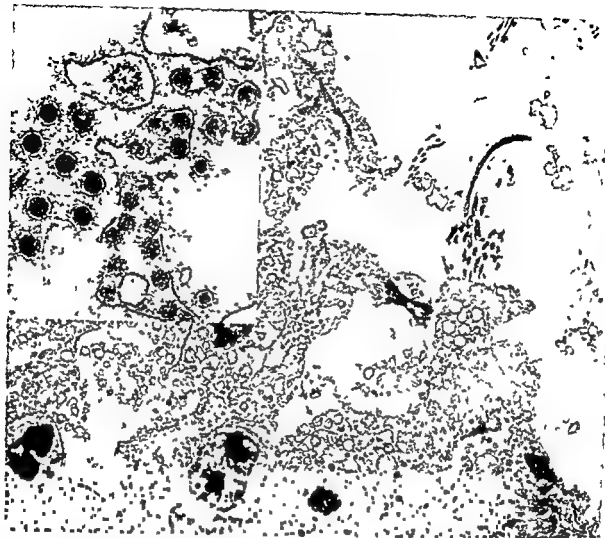


Fig 9 Electron micrographic survey of epithelial disruption similar to that in figure 8 The top of a hair cell can be seen in which the relationship to the reticular lamina is maintained The inset shows a cross

section of a sensory hair bundle in this region Note how the central cores of the stereocilia have retracted away from the surrounding limiting membrane ($\times 5000$ Inset $\times 43000$)

the structure of the hairs is altered This is shown in the inset where the central cores of the stereocilia seem to have become shrunken and separated from the surrounding limiting membrane In one region the cores of several stereocilia appear to be in the process of being surrounded by a common membrane The membrane around the kinocilium is also distended, but the internal fibrils are still normal in appearance

The basement membrane is usually found to be intact, with some fragments of supporting cell cytoplasm and membrane adherent to

it However, in other areas all epithelial structures are completely gone (Fig 10) Severe cryosurgical changes can also be seen in the subepithelial regions where the vessels are congested, although the endothelial lining is intact In the myelinated nerve fibers there is degeneration of mitochondria, shrinking of axoplasm, and disruption of the myelin sheath

Fig 11 shows the secretory cell region from the side of an ampulla in which the sensory cells have been completely destroyed The dark cells are surprisingly normal appearing when compared with the surrounding chaos As can

be seen the pigment cells below the dark cells are completely destroyed with free pigment granules dispersed in the connective tissue. The mitochondria and cytoplasmic processes are normal appearing and active pinocytosis can be seen at the endolymphatic surface. The nucleus is as in the normal state somewhat irregular (Kimura et al 1964).

Effect of survival time after cryosurgery

Very little difference is discernible on light microscopy between a specimen taken immediately after cryosurgery and one taken two hours later (Figs 12a and 8a). The amount of epithelial fragmentation and vacuole formation is very similar in both instances. These findings are confirmed on electron microscopy.

In specimens taken 1 day after the cryo-

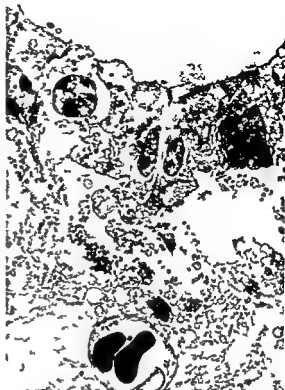


Fig 11 Electron micrograph of the dark cell area from a crista in which the sensory epithelium had been destroyed. The pigment cells are disrupted but the dark cells are intact. The mitochondria and cytoplasmic processes are normal appearing ($\times 1400$).



Fig 10 The sensory epithelium has lifted away from this portion of ampullary crista leaving an exposed basement membrane. Note the destruction of mitochondria in nerve axons and the disorganization of myelin sheaths in the connective tissue ($\times 1300$).

surgical exposure (Fig 12b) it can be seen that the epithelial debris is beginning to "float away" from the connective tissue of the crista and the remaining viable part of the sensory epithelium has tapered down to even out the cristal surface. Electron micrographs of this region show that it is the supporting cells that are flattening down to give this appearance.

After 4 days a thin cell layer has again covered the connective tissue (Fig 12c) in which the vascular and neural elements have been replaced by a fibrous substance. An electron micrograph of this region (Fig 3) shows the healing cell layer to be composed of greatly flattened and elongated supporting cells. A thickened basement membrane lies between these cells and the fibrinous deposit below.



Fig 12 Light micrographs of lateral ampullary cristae taken from animals that survived 2 hours (a) 1 day (b) and 4 days (c) after cryosurgery (-75°C probe). There is little difference between the 2 hour specimen and one taken immediately after rewarming (Fig 8). There is an area of demarcation between the cellular debris and viable epithelium in Fig 12b and after 4 days this debris has disappeared and the crista has healed (Toluidine blue, $\times 200$)

Some giant hairs can be seen in a few of the remaining hair cells on the side of the crista opposite the major damage in this same specimen (Fig 14)

Utricular changes

At vestibular temperatures of $+10^{\circ}\text{C}$ the utricle still appears to be morphologically intact. Even in the preparations where the lateral

canal ampullary crista was completely destroyed only very minimal changes were ever visible in the utricular epithelium by light microscopy. When this specimen was examined by electron microscopy moderate mitochondrial changes plus a few cytoplasmic vacuoles could be seen. However in some cases where there was relatively severe damage to the lateral crista (Fig 7) the utricle was completely nor



Fig 13 Four days following the cryosurgery the basement membrane is seen to be covered by a layer of flattened supporting cells. These are felt to migrate in

from the periphery of the lesion. The basement membrane is slightly thickened and below it there is an increased amount of fibrinous material ($\times 6900$)

mal on both light and electron microscopy (Fig 15). The possible reasons for this remarkable utricular resistance will be discussed in the next section.

DISCUSSION

The various morphological effects of cryosurgery on the guinea pig vestibular labyrinth have been presented through the range of minimal changes to severe epithelial disruption. Our findings suggest that the main disruption occurs immediately after the cryosurgical exposure. As demonstrated in a previous paper (Morrison & Lundquist, 1973) many of these epithelial changes can be produced without actually cooling the epithelium to the freezing point, in fact moderately severe intracellular damage can be done to hair cells when they are cooled to only $+18^{\circ}\text{C}$ or $+19^{\circ}\text{C}$. As has been shown, the earliest changes in-

clude destruction of the internal cristae of the mitochondria and vacuolization of the hair cell cytoplasm. These changes then progress to pyknosis of nuclei and general epithelial fragmentation. Following this degree of destruction the disorganized epithelium becomes separated from the underlying connective tissue, and within 4 days the cellular debris has been cleared away from the ampullary region.

Within the sensory epithelium the hair cells and nerve endings are most susceptible to cryosurgical damage, and the supporting cells less so. The dark cells of the secretory region also seem less susceptible to damage than other cells around them, such as pigment cells or the hair cells.

The changes present are similar to those found by Wersall et al (1965) in a study of postmortal degeneration. However, in that investigation it was stressed that the mitochondrial disturbances first appeared in the nerve



Fig 14 Giant hair found on the less affected side of the specimen shown in Fig 12c, taken 4 days after cryosurgery ($\times 16\,400$)



Fig 15 Normal type I and type II hair cells in a utricle that had been cooled to $+20\text{ }^{\circ}\text{C}$ ($\times 2\,500$)

endings because of their higher metabolic rate, in contrast to cryosurgical damage in which the changes are first seen in the apical cytoplasm. In both cases the degeneration is probably a result of a metabolic block, but in the cryosurgical specimens the nerve endings are shielded from the cold.

That the dark secretory cells are comparatively undisturbed could be explained by their position close to the protective vascular network of the bony ampullary wall.

The severe cryosurgical trauma that leads to epithelial disruption also affects the basement membrane to some extent, and it is interesting to find that some pieces of cytoplasmic membrane are attached to the basement membrane residues. This might lend support to the theory that the basement membrane is produced by the epithelial cells themselves, rather than the connective tissue (Pierce, 1965; Eriksson & Frithiof, 1973).

After 4 days however, the denuded areas are again covered by an epithelial lining consisting of supporting cells resting on a once again intact basement membrane. In the supporting cells of other animals, particularly the squirrel monkey, striated inclusion bodies can be found close to the centrioles, which might be interpreted as sign of capability of cell division, as has been suggested by Spoendlin (1966). In the present study no evidence of cellular division was found, and the manner in which the supporting cells are stretched out over the basement membrane suggests that they have not been newly formed. An active migration of these supporting cells from the wound edges is thought likely to occur. Further investigation of this healing process is underway utilizing scanning electron microscopic techniques.

As mentioned above the utricle is remarkably resistant to cryosurgical destruction. There are several possible reasons for this. Firstly,

the bone against which the utricle lies is on the opposite side of the vestibule from the lateral and superior ampullae so that it would be less affected by conduction of the cold through bone. Secondly, the cooling front arriving at the utricular region must cross the inner ear fluids, and as a result may be dissipated in various directions. Also, the epithelial surface of the utricle is protected to some extent by the otolithic membrane which may act as a blanket, isolating the epithelium from the temperature gradient. It has been shown by Stahle (personal communication) that the blood supply below the utricular epithelium is quite extensive, more so than in the connective tissue of the ampullary cristae. This would give the circulatory system a better chance to keep the utricle warm during the cryosurgical procedure.

From the present investigation we would suggest that the main effects of cooling on the vestibular epithelium are the result of a metabolic block, and not caused by actual freezing or ice crystal formation. The tissue can, of course, be frozen if lower temperatures are used and these effects have been shown by Lundquist et al (1972).

ACKNOWLEDGMENT

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ZUSAMMENFASSUNG

Die Wirkung der Kryochirurgie auf das Labyrinth des Meerschweinchens wird nach Licht- und elektronenmikroskopischer Untersuchung dargestellt. Das untersuchte Material stammte von Tieren, bei welchen die intralabyrinthären Temperaturen bekannt waren (Morrison & Lundquist 1973 im Druck). Bei Temperaturen zwischen $+11^{\circ}\text{C}$ und $+19^{\circ}\text{C}$ treten Veränderungen zuerst an den Mitochondrien im apikalen Teil der Haarzellen nahe der Endolymphe auf. Bei niedrigeren Temperaturen können mitozytoplasmatische Vakuolen und Verklumpungen von Kernchromatin beobachtet werden bei fortschreitenden Veränderungen werden Stützzellen sekretorische Regionen und auch das Bindegewebe betroffen. Bei noch niedrigeren Temperaturen ($+4^{\circ}$ bis $+10^{\circ}\text{C}$) kommt es zur Fragmentation und Abhebung des

Epithels vom Bindegewebe. Diese Zelltrümmer werden jedoch innerhalb von 4 Tagen beseitigt, und die Crista verheilt mittels einer Schicht von abgeflachten Stützzellen.

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Fig 14 Giant hair found on the less affected side of the specimen shown in Fig 12c taken 4 days after cryosurgery ($\times 16\,400$)

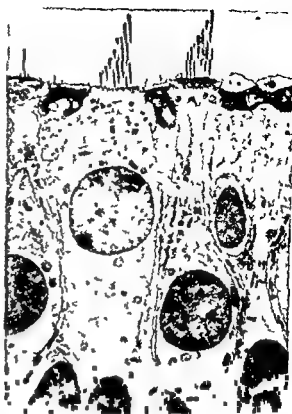


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ERRONEOUS PERCEPTION OF VERTICAL MOTION BY HUMANS SEATED IN THE UPRIGHT POSITION

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Abstract The reliability of subjective response to vertical linear accelerations in the absence of vision has been examined in human subjects seated in the upright position. Helicopters and a vertical movement simulator were used to generate sinusoidal vertical movement of period 2-10 sec and acceleration amplitude 0.2-0.4 g. Subjective response was recorded as a continually variable assessment of vertical position. Without prior knowledge of the movement pattern, subjects were aware of movement but registered its form with a performance little better than chance. Light to moderate simulated "turbulence" did not significantly alter performance. Detailed consideration of end-organ orientation in the skull suggests the possibility that relative paucity of vestibular afferent information about vertical movement may account, at least in part for the poor tracking performance observed in these experiments.

The quoted thresholds of human sensation to linear acceleration appears to be in the order of 0.005 to 0.01 g (Jongkees & Groen, 1946, Walsh, 1961, 1962 and 1964, Meiry, 1965, Young & Meiry, 1968). However, pilot experience in aircraft, especially helicopters and VTOL aircraft (Malcolm, 1971) suggests that the perception of vertical motion (i.e. parallel to the gravity vector) in an erect, seated, position is in practice considerably less effective than would be expected from this order of sensitivity. Possibly this apparent discrepancy might arise from the fact that none of the investigations cited above used

specifically vertical accelerative motion with the subject seated in an upright position.

Horizontal accelerations, whether parallel or transverse to the body's long axis, introduce changes in direction of the resultant linear acceleration vector due to summation of horizontal and gravitational accelerations, and this has been shown of itself to generate significant physiological response (Guedry, 1965, Benson & Bodin, 1966, Niven et al., 1966, Benson et al., 1970). Walsh (1964) studied response to vertical motion but the long axis of his subjects was horizontal. As discussed later, the anatomical arrangement of the vestibular organs in the skull could well lead to discrepancy between results obtained in this way and similar ones obtained with a subject seated erect. The following experiments were therefore designed to investigate the ability of human subjects to track purely vertical accelerative motion when seated erect.

METHODS

The experiments were conducted in three phases, of which the first and second employed helicopters in real flight and the third used the NASA Height Control Apparatus (HCA) with the generous co-operation of NASA Ames Research Center.

This research was supported by Canadian Defence Research Board Grant Numbers 9910-37 and 9310-92.

Phase one (Preliminary Experiment)

Ten male military personnel, five of whom were aircrew, were seated in the back of a large transport helicopter. They wore blind folds and were asked to signal the direction in which they felt they were moving by means of pre arranged hand signals. Using manual control, the pilot of the helicopter tried to approximate vertical sinusoidal oscillations having peak to peak accelerations of 0.4 to 0.6 g, that is, well in excess of the threshold values referred to above. He was asked to perform 20 cycles each of oscillations having periods of 2, 4, 6, 8 and 10 sec/cycle. An onboard observer maintained visual contact with the ground and surrounding nearby hangars, and by means of the same kind of hand signals as the subjects indicated the actual motion of the helicopter. The entire procedure was filmed on 16 mm cine film for later analysis.

The subjects had been told that they would experience random motions representing different types of turbulence. The films revealed that they quickly got out of phase with the true motion of the machine, some even reporting that they were moving in precisely the opposite direction to that of the real helicopter motion!

These surprising preliminary findings obviously warranted further investigation with better methods of applying the stimulus movement and of recording the human response.

Phase II (Computer Controlled Helicopter)

The National Aeronautical Establishment of the National Research Council of Canada provided the services of a helicopter which could be completely controlled by an onboard analogue computer.

Subjects Useful data were obtained from 6 volunteers who were employees of the National Aeronautical Establishment. Ages ranged from 21 to 40 years, and 3 of the subjects were current pilots, 2 were women.

Stimuli The computer was programmed to fly the helicopter through 20 cycles in each of five runs having successive periods of 2, 4, 6, 8 and 10 seconds. Each subject was exposed to all five runs. The accelerations of the helicopter, as measured by its onboard accelerometers, closely approximated the intended sinusoidal pattern. The peak-to-peak acceleration in all the runs was 0.4 g. The nature of the control program was such that the sinusoidal oscillations were superimposed on a steady rate of ascent. This latter was not detectable by the subjects, and no evidence could be found that it influenced the data.

Data acquisition As in Phase I, the subjects were told that the motions of the helicopter would be random vertical movements attempting to approximate different types of turbulence. The helicopter accommodated one subject at a time along with the pilot who controlled the experiment. The subject was equipped with a signalling device strapped to his right thigh as illustrated in Fig. 1. This device consisted of a box from which protruded two handles, one fixed (to act as a reference) and the other free to move back and forth through 90°, corresponding to a handle travel of some 5 to 6 inches. By means of this lever, the subjects were asked to indicate their perceived altitude. They were to move the handle forward if they felt they were high with respect to the starting point, and back towards their stomachs if they felt they were low. The lever was attached to a potentiometer battery combination which produced a bipolar analogue voltage proportional to the displacement of the handle. This signal was then fed to the helicopter's flight recording apparatus, along with the outputs from the onboard accelerometers. Fig. 1 shows one of the subjects operating the signalling device while seated in the helicopter.

Special conditions A considerable amount of noise was generated by the helicopter's motor and rotors, as well as by aerodynamic noise

from the cockpit walls. To prevent a subject from gaining possible cues from this noise, he wore a pair of highly attenuating earphones (Sharpe HA 10, Mk 2), which were driven by two loud audio tones. One tone was fixed in frequency at 1 kHz while the other tone varied in pitch according to the position of the signalling handle. When the handle was in the center position, the tones were adjusted so that they were identical, and when the subject signalled he was high, one of the tones would increase in frequency accordingly, and vice versa. The apparatus used to generate these tones was contained in a second box strapped to the subject's other thigh. The subjects kept their eyes tightly closed at all times during the runs.

Phase III (NASA Height Control Apparatus)

As will be seen below, the results of the Phase I and II experiments were quite unexpected. Possibly the subjects were being confused by disturbances in the stimulus caused by turbulence. In order to investigate this aspect of the problem in greater detail, as well as to expand the data field of the helicopter experiments, another experiment was performed on the Height Control Apparatus (HCA) at NASA's Ames Research Center in California. This device is an enclosed aircraft cockpit, without windows, mounted on runners and wheels such that it can move up and down a vertical track which is fixed to the side of a large building. The HCA is controlled by a computer, and is capable of moving smoothly through a vertical displacement of 80 ft with a 10 ft safety margin at each end.

Subjects The subjects used in this phase were two women and eight men, ranging in age from 27 to 49 years, none of whom was a pilot.

Stimuli The subjects were again exposed to 20 cycles each of oscillations having successive periods of 2, 4, 6, 8 and 10 sec/cycle.



Fig 1 Subject seated in helicopter operating handle of signal box. Two loud tones in the earphones masked noise cues associated with the motion.

In this series however, the peak-to-peak amplitude of the accelerations was 0.2 g. This somewhat lower value was necessitated by the limited travel available on the simulator as compared with the helicopter. However, it is at least an order of magnitude higher than the threshold values quoted in the introduction.

Two different levels of turbulence were simulated on the HCA. A sample of accelerometer records from these as well as the control runs is shown in Fig 2. The control stimuli (top trace) provided the smoothest movement available on this device. Light turbulence was simulated by superimposing on to the driving function of the control computer a noise signal whose power spectrum had a gaussian distribution centered at 8 Hz. The resultant accelerometer record is shown as

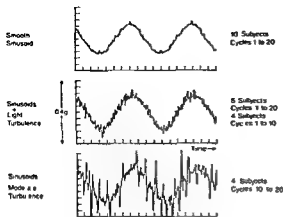


Fig 2 Samples of accelerometer records from the Height Control Apparatus at NASA Ames Research Center

the center tracing of Fig 2. A moderate degree of turbulence was simulated by introducing random electrical pulses of short duration which would cause the cab of the HCA to lurch up or down as shown in the lowest trace of Fig 2. It is worth emphasizing that the acceleration records in Fig 2 are displayed at relatively high gain. In practice, the perturbations seen on the top trace are barely perceptible, whilst the bottom trace is indeed equivalent to moderate turbulence.

All subjects were exposed to the smooth sinusoids (control runs) as shown in the top trace of Fig 2. They were then divided into two groups. One group of five repeated the control experiment to which had been added the light turbulence, and the second group of four (one man being unavailable) was subjected to the moderate turbulence during the last half of each run.

Data acquisition The subjects were asked to signal their perceived height with respect to the starting point of each run with the movable lever system described above. This time, however, the box was mounted on a vertical column fixed to the floor of the cab and positioned between their knees. This allowed the lever to be moved up and down instead of fore and aft, an arrangement felt to be

more closely allied to the real vertical motion. The subjects were instructed to rest their right forearm on their right thigh, in order to minimize the introduction of artifacts due to inertia of the arm. The subject's responses were fed by cable to a pen recorder, along with signals proportional to the acceleration, height and velocity of the cab.

Special conditions The earphones and tone generators were used again in order to minimize extraneous auditory cues. The subjects seated upright in an aircraft seat with shoulder and lap restraint. They were instructed to keep their eyes closed throughout the runs.

Unfortunately, a condition for using the HCA required the experimenter to show the subjects the exact nature of the stimuli they would be exposed to. It was later realized that the subjects' knowledge of the periodic nature of the motion may have significantly altered the manner in which they responded to the stimuli, a matter which will be discussed at greater length below.

RESULTS

Phase II

A sample of the original data obtained from one subject during the computer-controlled helicopter phase of the experiment is shown in Fig 3. The upper trace (1) is a record from the accelerometer which was sensitive to the vertical acceleration of the helicopter.

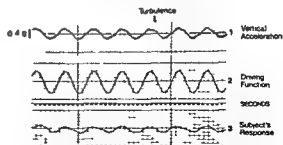


Fig 3 Sample of raw data obtained from a subject in the computer-controlled helicopter. The distortion in the sinusoidal pattern labelled turbulence was unintentional. Note how the subject "tracks" this perturbation.

Trace (2) is a record of the sine wave voltage used as the forcing function to drive the helicopter's flight control computer. Trace (3) is a record of the subject's tracking response. In this example, the subject was tracking the real movement rather well, but as will be seen below, this was by no means typical. Note how, when the acceleration record was distorted by turbulence, the subject quickly signified that something had happened, indicating that he was concentrating on tracking the motion of the helicopter. This degree of concentration was typical of all subjects. Additional traces of rates of yaw, pitch and roll, not shown here, confirmed that computer stabilisation in these three degrees of freedom effectively eliminated any cross-coupled aircraft motion.

These data were reduced by measuring the delay of the subject's peak response with respect to the peak of the stimulus. From this, was calculated the phase angle of the peak.

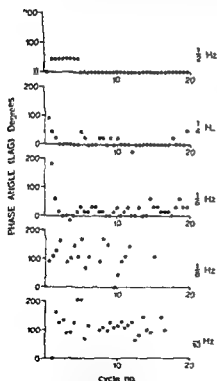


Fig 4 Phase angle (lag) of the subject's response as compared with his actual position in space. There is one dot for every half cycle of response.

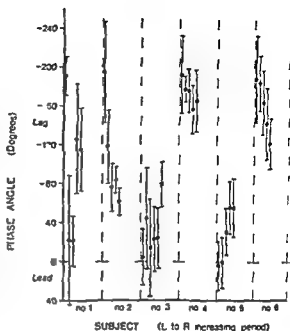


Fig 5 Average phase angle of the subject's response as compared with their actual position for all subjects and all runs. Each dot represents the mean phase angle averaged over the twenty cycles of each run. Range bars indicate one standard deviation. For each subject displayed along the horizontal axis the period of the motion is shown increasing from left to right from 2 sec to 10 sec.

responses with respect to the peak stimuli for each cycle of each run. Fig 4 shows graphs of the phase angles of a subject's responses for each of the five periods to which he was exposed. In this and all other graphs plotting phase, a negative sign on the ordinate indicates phase lag of response relative to stimulus. In this subject's responses, there was a tendency for increasing phase lag to be associated with decreasing frequency. However, the change with frequency was not consistently progressive. Moreover, no individual subject was representative of the whole group.

Fig 5 gives the average phase angle for an entire run (20 cycles) as a function of the period of the oscillation for each of the six subjects tested. The data for each subject are separated into six vertical columns by the dashed lines. Within each column the mean phase angle and its standard deviation are plotted from left to right in order of increasing period.

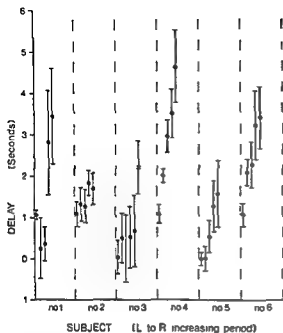


Fig 6 Average delay of each subject's response for each run. As in Fig 5, the dots represent the means over twenty cycles, while the bars indicate one standard deviation. The data are displayed in the order of increasing period of oscillation along the horizontal axis for each subject.

On visual inspection of Fig 5, it can be seen that there was apparently little or no consistent change of phase with frequency from one subject to the next and this is confirmed by the absence of statistically significant trends between subjects. However, in the figure it can be seen that there was a tendency for the results from subjects number 3, 4, 5 and 6 to cluster round the phase angles 0 or 180°. This cannot be said of the results from subjects number 1 and 2. The implications of these two features will be discussed later.

The points depicted by an X in this and the following figure (6) are the results of asking one subject to repeat the run having a period of 10 sec while keeping the eyes open. The sky was quite clear, with unlimited visibility. The average height of the helicopter was 1500 ft above an open field, yet this subject's mean phase error during this run was

almost twice as great as that obtained from the same stimulus with eyes closed only minutes earlier. It should be noted that during this time, the helicopter underwent changes in altitude of the order of 200 ft!

Fig 6 is derived from the same data as was the previous figure and shows the average delay of the peak response relative to peak stimulus as a function of the period of the motion. Once again, each dot corresponds to one subject's results averaged over the 20 cycles of a run at a given period.

Upon inspection, it is obvious that a consistent pattern of delay cannot explain the overall results. On the one hand, subject number 2 manifested a relatively constant delay in responding to all stimuli, whereas subject number one showed a widely varying delay which cannot even be attributed to a systematic change in phase angle. The pattern of delays in the responses from subject number 4 conforms with those responses being consistently about 180° out of phase with the stimulus.

Phase III

Data from the phase 3 experiments conducted on the Ames Research Center Height Control Apparatus (HCA) were reduced in the same manner as those from the helicopter.

Fig 7 shows the phase angle of response as a function of the period of the oscillation for all the runs on the HCA. Each point represents the average response of a subject to 10 cycles at a given period of oscillation. The open circles are derived from the smooth runs, the closed circles from the light turbulence runs and the triangles from the moderately turbulent runs. As can be seen, no significant effect on the subject's responses can be attributed to the turbulence. Also of interest is the relatively large proportion of subjects who anticipated the motion of the cab, as signified by the responses which lead the stimulus.

The data offer a means of assessing the reliability of one's perception of motion un-

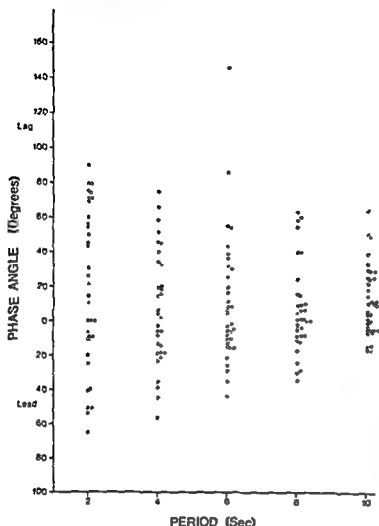


Fig 7 Average phase angle of subjects' responses compared with position of Height Control Apparatus as a function of the period of the oscillation \circ data from the smooth runs Δ , light turbulence runs, ∇ , moderate turbulence runs. Each point represents the mean of ten cycles

der the conditions of this experiment. In the computer-driven helicopter, 586 subject-cycles were completed, and 281 (48%) were out of phase by more than 90 degrees. If, during the run, a subject could not make up his mind which way he was moving, he generated a "blank" response by centering the handle of the signal box and holding it steady. Of the responses from the helicopter, 27 (4.6%) were "blank".

In the HCA, 1943 subject-cycles were completed. Of these, 153 (7.9%) were out of phase by 90° or more, 466 (24%) were out by 45° or more, while 301 (15.5%) were "blank". These "blank" responses were predominantly the result of two subjects who became "lost" for the majority of the experiment. If these

two had reacted with the same assurance as the other subjects on the HCA, the proportion of "blanks" would be similar to that found in the data from the helicopter.

DISCUSSION

An important outcome of these experiments is that, although subjects usually made well-defined oscillatory tracking responses and hence presumably thought they knew what was happening, in practice those responses were likely to be quite wrong. It is difficult to decide whether the errors were due to a low signal-to-noise ratio from the senses involved, or to a specific liability to make an incorrect assessment of the direction of move-

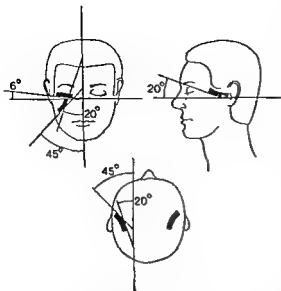


Fig 8 Planes occupied by the gravity receptor organs. Free after, Quix (1923) Müller (1962) and Jongkees (1967)

ment. Thus in Fig 5, the widely scattered phase data from subjects 1 and 2 suggests the problem was one of resolving signal from noise, whilst the results from subjects 3, 4, 5 and 6 suggest it may have been largely one of determining the correct direction of move-

ment. Both these possibilities could account for the occasional "blank" responses representing frank admissions by the subjects that they were confused.

These findings are in accord with those of Correia et al (1968), who noted relative insensitivity of the oculogravic illusion (Graybiel, 1952) to linear acceleration along the body's long axis, especially when the subject's head was tilted forwards 30° as was the case in our experiments. On the other hand our results from vertical movement stimuli contrast markedly with corresponding results obtained by Meiry (1965) and Young & Meiry (1968) from subjects exposed to sinusoidal linear accelerations in a horizontal plane. The responses of their subjects were sufficiently consistent to define characteristic changes in phase of response with frequency of sinusoidal stimulation, from which a stimulus response transfer function was deduced.

It should be emphasised that in all these experiments the subject's whole body was exposed to the varying force field which constituted the stimuli. Hence it is quite possible that different patterns of response could be accounted for by subjects responding to



Fig 9 Plastic model showing the spatial relationship of the gravity receptor organs. Large arrow points in the forward direction and lies in the horizontal plane defined by the axes in Fig 8. Small arrows seen on the model indicate presumed direction of maximum excitability of sensory cells in the organs (see text)

different sets of sensory inputs, ranging from the special acceleration sensitive organs of the vestibular system to superficial and deep sensations throughout the body.

However, this possibility is rather difficult to reconcile with Walsh's observation (1961) that the sensation threshold of Labyrinthine Defective (L-D) subjects to sinusoidal linear accelerative stimuli was an order of magnitude higher than that of normals. Indeed, the relative importance of vestibular sensation in determination of response to accelerations has frequently been emphasized by the impairment of such response in L-D subjects (Miller & Graybiel, 1971, Graybiel & Fregley, 1966, Nashner, 1970). One might therefore surmise that the vestibular signal would predominate, especially in marginal conditions of uncertainty, which in turn points to the vestibular system itself as a likely source of the specific difficulty which appears to be associated with sensing vertical motion.

Following this lead, Fig. 8 shows the approximate orientations of the gravity (linear acceleration) receptor organs in man, derived from Quix (1923), Miller (1962) and Jongkees (1967). It can be seen that each organ is curved in such a way that its surface has components in all three spatial planes. This feature can be better visualized in Fig. 9, which is a photograph of a plastic model demonstrating the relative spatial dispositions of the planes of the gravity receptor organs. The large arrow points in the forward direction and lies in the horizontal plane defined by the axes in Fig. 8.

It has been shown that the receptors are stimulated when the relatively dense otoliths glide over the sensory epithelia which occupy the planes seen in Figs. 8 and 9 (Trincker, 1962). Each receptor cell has a direction of maximum excitability, this being the direction of shear of the otolith which causes the greatest relative increase in the action potential frequency of the subsequent neuron (Lowenstein & Wersall, 1959, Flock & Wersall, 1962, Spoendlin, 1966). The small arrows

in Fig. 9 indicate these directions of presumed maximum excitability of the receptor cells based on histological data from the guinea pig reported by Spoendlin in 1966, although some uncertainty exists as to the precise orientation of each organ relative to stereotaxic coordinates of the skull (Spoendlin, Personal Communication).

From Fig. 9, it is apparent that, providing the above interpretation is correct, then vertical acceleration will generate little response from the utricular organs since these lie close to a horizontal plane and hence the small arrows have only a minimal projection in a vertical direction. Rather surprisingly the model in Fig. 9 indicates that even in the saccular organ there is only a relatively small projection of small arrows in a vertical direction. Thus, it seems quite possible that paucity of vertical representation in the end-organ could be largely responsible for the particular difficulty attached to sensing body motion in a vertical direction, although of course central processing could also be at play.

From an applied view point, the present results have an obvious bearing on the control of helicopters and VSTOL aircraft. Thus from the helicopter experiments it appears that an unaided individual (i.e. no access to instrumentation) exposed to vertical motion without prior knowledge of its nature and direction, has no better than a 50% chance of "guessing" the direction of motion correctly and a high probability of being wildly out of phase in his assessment. Furthermore from the trial using clear outside vision (but still no access to aircraft instrumentation) it seems that above some 1500 ft, normal visual cues about vertical motion are too weak to improve the situation.

Presumably in normal man-controlled flight the difficulties must be much reduced by the fact that a pilot flying an aircraft anticipates his movement according to his control action. Indeed in the HCA experiments when subjects had to be shown the precise nature of their stimuli before experimentation per-

formance was better than in the helicopter experiments, when subjects were falsely informed that they would be exposed to random patterns of vertical motion. However, even in the HCA experiments there was no consistently reliable pattern of response, which further highlights the above implications concerning instrumentation.

ZUSAMMENFASSUNG

Die Richtigkeit subjektiver Lageempfindungen bei vertikalen, linearen Beschleunigungen wurde an Versuchspersonen mit verbundenen Augen und in aufrechter Sitzhaltung untersucht. Mit Hubschraubern und einem Vertikalbewegungssimulator wurden sinusförmige Vertikalbewegungen von 2-10 Sekunden Periodendauer und mit einer Beschleunigungsamplitude von 0.2-0.4 g erzeugt. Die Bewegungsempfindungen wurden als ständig variable Beurteilung der Vertikallage registriert. Die Versuchspersonen, die über die Art und Richtung der Bewegungsabläufe nicht unterrichtet worden waren, bemerkten wohl die Bewegung selbst, konnten aber ihre Form nur mit einer wenig mehr als zufälligen Genauigkeit erkennen. Eine leichte bis massige, Turbulenz-Simulation brachte keine signifikanten Änderungen dieser Sachlage mit sich. Eine eingehende Betrachtung der Ausrichtung der Nervenendorgane im Schadel legt die Vermutung nahe, dass das unverlässliche Orientierungsvermögen der Versuchspersonen zumindest zum Teil durch die verhältnismässig geringe Anzahl der durch vestibuläre Affferenz übertragenen Reizinformationen über vertikale Bewegungen verursacht wird.

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SQUIRREL MONKEY STRAIGHT PLATFORM RUNWAY

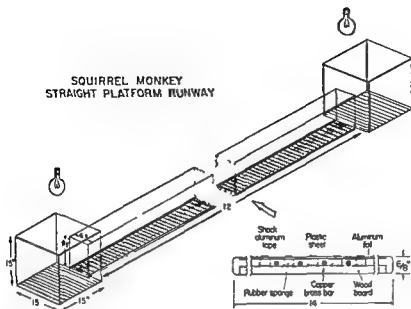


Fig 1 This schematic drawing shows the structural arrangement of the present squirrel monkey platform runway (straight) device. Photocells (which are not illustrated in this figure) are installed at junctions between shuttle boxes and runway.

Apparatus

The apparatus for the squirrel monkey platform runway test consists of two 15" cubical shuttle boxes connected by a 14" wide, 12' long, straight platform which is covered with a proper-sized enclosure (Fig 1). Scrambled electric shock grids are mounted on the floor of each shuttle cage. Over each shuttle cage is a light bulb which provides a discriminating stimulus. On the floor of the platform runway, except for a 2" wide center line, 12 channels (6 channels on each side) of pressure sensor type recording system are installed. The channels are activated when a squirrel monkey steps on them. These pressure sensors are covered by a thin plastic sheet to prevent contamination, over the plastic sheet, shock aluminum tapes are installed along its longitudinal axis. These shock tapes are used during the training stage to shape the subject's performance to walk straight on the center strip. The entire device is painted black so that the subject can recognize the target (lighted) shuttle box easily. Through a small, unpainted area of the enclosure ceiling, the examiner can observe a part of the subject's walking performance.

Cumulative counters are used to record the

number of channel activations in order to examine the direction and degree of dysequilibrium, an event recorder is used to print out the subject's gait performance. Crossing time for each trial run is presently measured by an electronic counter which is activated by photocell relays.

Subjects

South American born squirrel monkeys (*Saimiri sciureus*) are used. Their suitability for this sort of behavioral conditioning task has been demonstrated in the squirrel monkey rail test. These subjects are one and one-half to two years of age, both males and females are used randomly. The body weights of the animals range from 550 to 750 g.

Procedure

The procedure which has been developed is as follows. The subjects are first screened to determine whether they are the proper subjects for this type of psychophysical task. All animals should have an avoidance performance rate of better than 90% for the entire trial sequence.

After such a contingency training period, the animals are subjected to 100 shaping trials.

daily for five continuous training days. During these 500 trials, the squirrel monkeys are trained to walk straight along the center line which connects two shuttle boxes. For this purpose, squeeze-in side shocks on the platform are used.

The squirrel monkeys are then processed to the preoperative baseline measurement stage. Five warm-up runs are permitted on every testing day before the 10 recording trials are conducted. These recordings are used to establish the preoperative baseline. The tests are performed twice a week.

The present criterion which determines the subject's readiness for the experimental procedure (operation, etc.) is less than five deviations (channel activations) in either direction over ten consecutive recording trials for three continuous testing days. Subjects usually meet this criterion within 8 to 12 testing days.

Postoperative measurements usually begin three to four days after the operation. The tests are performed twice a week until the subject's bodily equilibrium performance returns to the preoperative status. The manner of daily data acquisition is exactly identical to that of the preoperative stage. Subsequently the animal is sacrificed by means of intracardiac perfusion and the temporal bones and related neural issues are removed and processed according to the standard preparation technique for morphological investigation.

REPRESENTATIVE DATA (LABYRINTHECTOMY)

Up to the present time seven squirrel monkeys have been assigned to the post labyrinthectomy (via retroauricular atticotomy approach) investigation. After unilateral labyrinthectomy, all subjects were ataxic (in varying degrees) but their avoidance performance rate which indicates the stability of conditioning did not change. More than 50 days (average 70-80 days) were required to regain the preoperative status of dynamic bodily equilibrium maintenance. The data were comparable to those obtained by the squirrel monkey rail test (Igarashi et al., 1970).

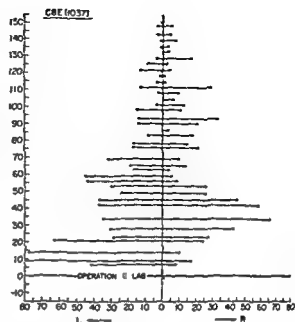


Fig. 2 This graph demonstrates the degree and direction of bodily dysequilibrium of one representative case after left labyrinthectomy. The vertical axis indicates the number of days before and after the operation. The abscissa demonstrates the total cumulative number of channel activations (toward subject's left or right) within ten trial runs of the day. X mark on the second postoperative day indicates the extreme difficulty of performance and discontinuation of data acquisition on the day.

Two cases needed more than 100 days to regain the preoperative status (Fig. 2).

The direction of ataxia was toward the operated side soon after the labyrinthectomy. In five cases out of seven in the present series, the direction of bodily dysequilibrium changed somewhat from one side to the other, while the number of channel activations gradually decreased from one testing day to another. Therefore, in some cases, at some time after unilateral labyrinthine lesion, the direction of ataxia was toward the non-operated side. The time period for one oscillation of this ataxic direction change varied, however, it was about three weeks in the present platform runway series. Previously, this oscillating tendency was noticed in some of the unilaterally labyrinthectomized monkeys studied with the squirrel monkey rail test (Igarashi et al., 1970). Two other cases in the present series failed to show such a tendency.

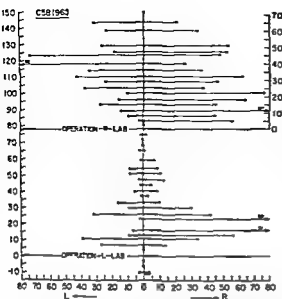


Fig 3 This graph also exhibits the dysequilibrium status of another representative after left labyrinthectomy and subsequent right labyrinthectomy. Compared to the previous case, which was demonstrated in Fig. 2, the degree of ataxia after unilateral labyrinthectomy was less and the compensation was faster in this case.

Some oscillating directional change of dysequilibrium was also seen on two occasions following the second labyrinthectomy (non simultaneous bilateral labyrinthectomy). Up to the present time, however, the majority of the subjects after the second labyrinthectomy have exhibited relatively dominant directionality toward the side of the second operation. It was found that recovery from dysequilibrium generally required a longer time after the second operation than after the first operation.

DISCUSSION

Compared to the squirrel monkey rail test which measures physically advanced maximum ability of dynamic equilibrium function, the present squirrel monkey platform runway test measures the degree and direction of dysequilibrium in an ordinary walk without being affected by physical training. In addition, an event recorder prints out the pattern of each trial run directly on a chart thus permitting subsequent analysis of directional compensation, etc. All

available parameters are collected for this subsequent analysis.

Platform crossing time is another important parameter which is being studied in order to analyze the equilibrium maintenance ability. Reduction of ataxic degree immediately after the operation (which can be seen in the present case demonstration) is due to the fact that the squirrel monkey usually slows down to traverse the runway. Thus, the crossing time should be included in the analysis of the ataxic degree.

Whereas the present behavioral physical task is not a difficult one, more squirrel monkey subjects can be accepted for the experimentation as compared to the squirrel monkey rail test. Also, by utilizing the squirrel monkey rail test, the quantitation of the low end of postoperative dysequilibrium was considerably poor. The severity of post-labyrinthectomy ataxia and post-utricular nerve section ataxia was "0" (maximum low score) for both categories when measured by the squirrel monkey rail test. By using the squirrel monkey platform runway test, the ataxic severity will be more properly compared among different experimental categories.

The results obtained from the present squirrel monkey platform runway test can be more reasonably projected toward the clinical application since the task procedure resembles the clinical balance test situation. Inasmuch as the total number of labyrinthectomy cases is still limited, no statistical results are presented in this communication. Representative cases only exhibit the general tendency of the post labyrinthectomy status.

After unilateral labyrinthectomy, the oscillating directional change of dysequilibrium was observed in some cases with the present squirrel monkey platform runway test. This tendency was previously noticed in some post labyrinthectomy cases in the squirrel monkey rail test (Igarashi et al., 1970). Similar to the bilaterally labyrinthectomized condition which was measured by the squirrel monkey rail test, equilibrium compensation after bilateral labyrinthectomy was found to be slow when measured by the squirrel monkey platform runway.

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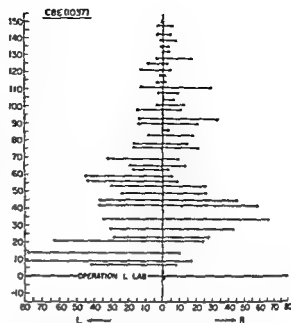


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MICROSPECTROPHOTOMETRIC DNA ANALYSIS OF MALIGNANT SALIVARY GLAND TUMOURS

C-M Eneroth and A Zetterberg

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Abstract Microspectrophotometric determinations of nuclear DNA quantity of Feulgen stained cells were made in six selected malignant salivary gland tumours and compared to normal salivary gland tissue and human lymphocytes from peripheral blood. Two questions of methodical importance were investigated and it was found that no errors in the quantitative DNA determination were introduced as a result of the imprint technique and that the optimal duration of acid hydrolysis in the Feulgen staining procedure was 60 min. Microspectrophotometric DNA analysis of the six malignant salivary gland tumours showed nuclear DNA quantities which exceeded that of the normal control cells irrespective of the type of tumor and degree of differentiation. Furthermore, a much higher degree of intercellular variability with respect to the nuclear DNA content was found in the malignant tumour cell populations. The fraction of cell nuclei with DNA values exceeding that of normal cells varied according to the type of tumour, and the incidence was small in biological low grade malignant tumours and high in high grade malignant tumours. Thus the nuclear DNA content seems to be an objective parameter in grading the malignancy of salivary gland tumours.

Abnormal chromosome number has been observed in malignant tumours both in experimental animals and in man (Koller, 1972), and the chromosomal abnormalities are often sufficiently pronounced to manifest themselves as an abnormal quantity of the nuclear DNA as determined by means of quantitative microspectrophotometry of Feulgen-stained cell nuclei (Leuchtenberger et al, 1954, Atkin et al, 1959, Atkin, 1964, Caspersson, 1964, Sandritter & Ritter, 1966, Böhm et al, 1971).

The salivary glands are the site of a number of different types of tumour of varying degree

of malignancy. Although salivary gland tumours have become more distinctly classified in recent years due to a clear histological definition, there may still be uncertainty concerning malignancy and prognosis and therefore also concerning the treatment of these tumours.

In order to estimate the malignancy of salivary gland tumours in a more objective manner than by an evaluation based solely on the morphological appearance, a comparative analysis has been made between tumours with different morphological features and their cytochemical properties. The aim of the present study was to investigate whether the morphologically heterogeneous group of malignant salivary gland tumours could be distinguished from normal salivary gland cells on an objective cytochemical basis and whether the degree of malignancy of the different types of tumour could be determined in the first place by using nuclear DNA quantity as cytochemical descriptive.

MATERIAL AND METHODS

Six cases of malignant salivary gland tumours were selected for analysis on the basis of the morphological features of aspirate from fine needle biopsy. For the histological examination of tumours, routine histopathological techniques were employed.

Immediately after surgical removal of the

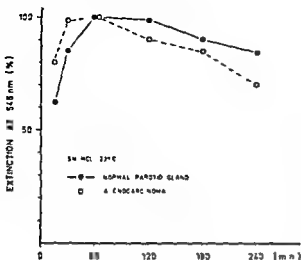


Fig. 1 Total nuclear extinction at 546 nm (amount of Feulgen positive material) plotted against time (min) of acid hydrolysis in 5 N HCl at 22°C. Filled symbols and solid lines represent cell nuclei from normal parotid gland tissue and the open circles and broken lines represent cell nuclei from a poorly differentiated adenocarcinoma (case 1, Fig. 2).

tumours, imprint preparations were made from the fresh cut surface through the tumour tissue. Haemacytometer glass slides were used. The imprint preparations were immediately fixed in a freshly prepared mixture of ethanol and acetone (1:1) for 30 min at room temperature and thereafter stored in a refrigerator (+4°C) until the staining was performed.

The DNA content of individual cell nuclei was determined after Feulgen staining by absorption measurements in a rapid scanning microspectrophotometer at 546 nm (Lomakka, 1965; Caspersson & Lomakka, 1970). The acid hydrolysis in the Feulgen staining procedure was performed in 5 N HCl at 22°C. The optimal duration of the acid hydrolysis was found to be 60 min (cf. Results).

Freshly prepared human lymphocytes from peripheral blood were used as control cells of the staining procedure on each occasion. All measured values were expressed in relation to each staining control, which was given the value 2.0 (cf. Results).

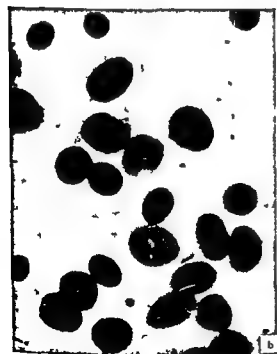
Stromal cells with elongated nuclei, lymphocytes and granulocytes could relatively easily be recognized in Feulgen stained preparations

and were not included in the measurements. Thus, only nuclei from tumour cells were randomly selected and measured.

RESULTS

Since the microspectrophotometric analysis of nuclear DNA quantity in the present study was based on the Feulgen staining reaction, it was considered to be of extreme importance to test the optimal staining conditions for the different types of cell to be analysed. For it has been shown that, in particular, the acid hydrolysis of DNA in the fixed cell nucleus is greatly affected by the state of the nuclear chromatin (Richards & Atkin, 1960; Sandritter et al., 1965; Kernell et al., 1971). The optimal hydrolysis conditions were therefore tested. Cells from normal parotid gland tissue were compared with cells from a low-differentiated adenocarcinoma. Fig. 1 shows the amount of stain bound to DNA (extinction at 546 nm) as a function of hydrolysis duration. The hydrolysis was performed in 5 N HCl at room temperature (22°C). The optimal duration of hydrolysis for the normal cells was between 60 and 120 min, while for the tumour cells it was between 30 and 60 min. 60 min was therefore considered the optimal duration of hydrolysis for all types of cell studied.

Another factor of great methodological importance, which had to be considered was whether the imprint technique introduced any artefacts for the DNA determinations. Since imprint preparations sometimes contain a relatively high frequency of "isolated" nuclei which might be more susceptible to the acid hydrolysis than were intact cells, imprint preparations made from mouse kidneys were compared with corresponding tissue culture preparations (primary mouse kidney epithelial cells) from parallel animals. The average DNA content of the imprint preparations was 10.8 ± 1.5 (mean \pm standard deviation) while for the tissue culture preparations it was 11.1 ± 1.8 (mean \pm S.D.). Thus no significant error with respect to the Feulgen DNA determination was introduced.



by the imprint technique as performed in the present investigation. The morphological appearance of the imprint preparations is shown in Fig 2: one poorly differentiated adenocarcinoma (Fig 2a), one adenoid cystic carcinoma (Fig 2b) and one mucoepidermoid carcinoma (Fig 2c).

The DNA content of the individual cell nuclei from the normal parotid gland tissue and from the six malignant salivary gland tumours is illustrated in the histograms in Figs 3 and 4. All histograms are based on randomly selected cell nuclei. At each staining human lymphocytes from peripheral blood were used as control cells and the mean DNA content of these cells was given the relative value of 20 C, which denotes the normal diploid nuclear DNA content. All other DNA values are expressed in such relative lymphocyte units. In this way preparations stained and measured on different occasions could be directly compared on a quantitative basis. It should be pointed out, however, that the measured differences between lymphocyte preparations stained on different occasions were quite small under the conditions used, with mean

Fig 2 (a) Adenocarcinoma (b) Adenoid cystic carcinoma (c) Mucoepidermoid carcinoma. Morphological appearance of imprint preparations. Photomicrograph 1400.

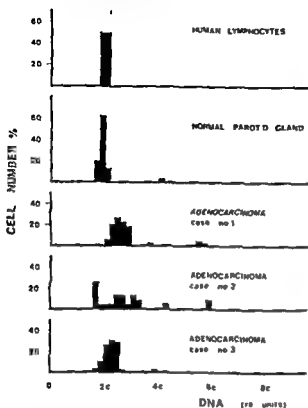


Fig 3 Histograms of nuclear DNA content (Feulgen positive material) of individually analysed cell nuclei in the rapid scanning microspectrophotometer. The cell number in each histogram class is expressed in per cent of the total number of cells analysed. Except for the lymphocyte preparations in which only 30 cells were analysed between 50-100 cells were analysed in each preparation. Adenocarcinomas 1 and 2 were poorly differentiated while adenocarcinoma 3 was moderately differentiated. The broken vertical line represents the upper limit of the normal diploid value.

values of 127, 128, 131, 131, 132 and 134 relative units of DNA on six occasions. As will be seen from Figs 3 and 4, cell nuclei from the normal parotid gland tissue contain the same amount of DNA as the lymphocyte nuclei. DNA values around 2.0 C i.e. the normal diploid amount.

In the six analysed cell populations from malignant salivary gland tumours (Figs 3 and 4) from 48% to 94% of the cell nuclei contained more DNA than the cell nuclei from the normal parotid gland, the upper limit of the normal DNA value (2.2 C) is indicated by the broken lines in the two figures. Some of the nuclei in the tumour cell populations contained as much

as 3 times the normal DNA amount. As is furthermore evident from the histograms in Figs 3 and 4, the overall intercellular variability in nuclear DNA amount is considerably larger in the tumour cell populations than in the normal cell populations. Despite this large DNA variability each tumour cell population was characterized by a modal DNA value in the region 2 C to 3 C, exhibited by a relatively large fraction of nuclei. In cases 1, 2, 3 and 4 (three adenocarcinomas and one adenoid cystic carcinoma) the modal DNA value was found in the hyperdiploid region, significantly larger than the upper limit of the normal diploid value (2.2 C). In cases 5 and 6 (two mucoepidermoid carcinomas) the

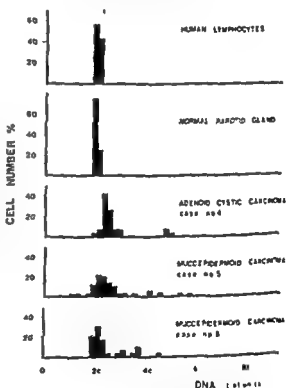


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lal DNA value was not found to be significantly larger than 2.2 C, although about half the nuclei in these two cell populations contained more DNA than that value. The tumour cell populations were furthermore characterized by calculating the fraction of cells with 'normal' DNA values, defined as less than 2.2 C. It was interesting to note that this fraction was very small in the poorly differentiated adenocarcinoma (case 1) and in the poorly differentiated adenoid cystic carcinoma (case 4), 6% and 9% of the cells respectively. For the well differentiated mucoepidermoid carcinoma (cases 5 and 6) the fraction was considerably larger, 40% and 52% respectively. Moderately differentiated adenocarcinomas (cases 2 and 3) had values in between, 33% and 40% respectively. An inverse relationship was thus found between degree of malignancy, as judged from the morphology, and the frequency of cells with 'normal' DNA values.

DISCUSSION

Questions of great methodological importance were investigated in the present study. Firstly, the optimal time of the acid hydrolysis of the Feulgen staining procedure was tested. Secondly, the effect of the imprint technique on quantitative DNA determinations. It was found that the hydrolysis curve of the chromatin of the tumour cells differed somewhat from that of the normal parotid gland cells. DNA was more rapidly hydrolysed in the tumour cell nuclei but was also more easily lost as the hydrolysis time was prolonged. Since the two hydrolysis curves overlapped an optimal duration of hydrolysis of 60 min could be used both for normal and tumour cells. It was furthermore found that imprint preparations made from mouse kidney showed the same nuclear DNA content as tissue-cultured mouse kidney cells from parallel animals. This indicates that the imprint technique as used in the present study, does not introduce any significant errors in the quantitative determination of nuclear DNA. The microspectrophotometric DNA analysis performed in the present study showed that the

cell populations from six malignant salivary gland tumours could be clearly distinguished from cell populations of normal salivary gland tissue.

The six malignant salivary gland tumours were selected so as to represent different degrees of biological malignancy.

The morphological features of poorly differentiated adenocarcinoma (case 1) and poorly differentiated adenoid cystic carcinoma (case 4) are connected with a high grade of biological malignancy (Eneroth, 1973; Eneroth et al., 1968), whereas the highly differentiated mucoepidermoid carcinoma (cases 5 and 6) are biologically low grade malignant tumours (Eneroth et al., 1972). Cases 2 and 3 represent adenocarcinomas not so poorly differentiated as case 1.

In all of the analysed malignant tumour cell populations a large fraction of cells, varying from 48 to 94%, clearly exceeded the upper limit of the normal diploid DNA value. Judging from the mitotic index, which for all tumour cell populations analysed was small (less than 0.1%), the overall growth rates of these tumour cell populations were low. Thus only a small fraction of the cells with increased nuclear DNA quantity could represent cells synthesizing DNA (S-cells) or having synthesized DNA (G2-cells) in the preparations for mitosis. Therefore the large majority of the cells with increased nuclear DNA quantity represent cells in the resting phase (G0) or pre-DNA synthetic phase (G1) of the cell cycle. The nuclear DNA determinations thus indicate the existence of aneuploid cells with raised chromosome number since a general correlation has been found between DNA content of tumour cells and their chromosome number (Richards & Atkin, 1960). Although some tumour cell nuclei contained as much as three times the normal diploid DNA amount, the majority of them only showed moderately increased DNA quantities. For the three adenocarcinomas and the adenoid cystic carcinoma, modal DNA values in the hyperdiploid region were found suggesting the existence of aneuploid stem lines with hyperdiploid chromosome numbers. For the two mucoepidermoid carcinomas, however, the observed modal DNA values were

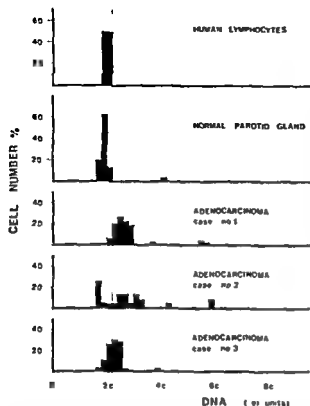


Fig 3 Histograms of nuclear DNA content (Feulgen-positive material) of individually analysed cell nuclei in the rapid scanning microspectrophotometer. The cell number in each histogram class is expressed in per cent of the total number of cells analysed. Except for the lymphocyte preparations, in which only 30 cells were analysed, between 50-100 cells were analysed in each preparation. Adenocarcinomas 1 and 2 were poorly differentiated while adenocarcinoma 3 was moderately differentiated. The broken vertical line represents the upper limit of the normal diploid value.

values of 12.7, 12.8, 13.1, 13.1, 13.2, and 13.4 relative units of DNA on six occasions. As will be seen from Figs 3 and 4, cell nuclei from the normal parotid gland tissue contain the same amount of DNA as the lymphocyte nuclei, DNA values around 20 C, i.e. the normal diploid amount.

In the six analysed cell populations from malignant salivary gland tumours (Figs 3 and 4) from 48% to 94% of the cell nuclei contained more DNA than the cell nuclei from the normal parotid gland; the upper limit of the normal DNA value (22 C) is indicated by the broken lines in the two figures. Some of the nuclei in the tumour cell populations contained as much

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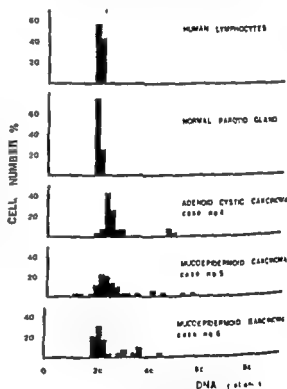


Fig 4 Histograms of nuclear DNA content (Feulgen-positive material) of individually analysed cell nuclei in the rapid scanning microspectrophotometer. The cell number in each histogram class is expressed in per cent of the total number of cells analysed. Except for the lymphocyte preparations in which only 30 cells were analysed, between 50-100 cells were analysed in each preparation. The adenoid cystic carcinoma case 4 was a poorly differentiated tumour with cylindrical structures and the two mucoepidermoid carcinomas were both well differentiated: case 5 (from the palate) and case 6 (from the submandibular gland).

SPRING MECHANISMS IN THE HUMAN LARYNX

II Raymond Fink

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Abstract Laryngeal movements are usually considered to be active in both phases of behavior cycles such as phonation, straining effort and swallowing. X ray, electromyographic and cinematographic evidence is presented suggesting that these cycles begin with an active phase of muscular contraction and end with a partly passive phase of elastic restitution. Elastic restitution may be a significant factor in the organization of laryngeal actions in general.

The changes of the laryngeal passage in phonation, straining effort closure, and swallowing closure are organized in cycles characterized by a phase of narrowing followed by a phase of widening. The succession of the phases is generally attributed to patterned activity of opposing voluntary muscles, little attention being paid to the role of adjoining elastic structures. For example, in a phonatory cycle, both adduction and abduction of the vocal folds are counted as active phases and passive contributions are disregarded (Stroud & Zwiefach, 1956, Greene, 1972). One may contrast this mode of succession with the present understanding of eupneic respiration, where a cycle is seen as consisting of an active phase, the inspiratory phase, followed by a largely passive phase, the expiratory phase of elastic recoil.

I submit evidence that it is the latter type of cycle—active displacement followed by largely passive restitution—that also underlies major behaviors of the larynx.

Supported by USPHS Research Center Grant GM 15991

METHODS

The evidence was obtained by means of X ray still and motion pictures, in three men and one woman, informed, consenting, and aged 30-42. Seven additional subjects were studied by cinefluorography only. Electromyography was performed in two of the subjects, using a Grass electroencephalograph. Concentric needle electrodes were inserted through skin wheals of 1% procaine hydrochloride as follows: sternothyroid and thyrohyoid muscles, electrodes advanced down to the thyroid cartilage respectively posterior and anterior to the tubercle at the caudal end of the linea obliqua, vocalis muscle, electrode inserted through the cricothyroid ligament, cricothyroid muscle, electrode inserted at the junction of the inferior thyroid cornu with the thyroid lamina and advanced down to the thyroid cartilage, rectus abdominis, electrode in the cranial third of the muscle deep to the aponeurosis, external oblique muscle, entered 2 cm above and medial to the anterior superior spine of the ilium. In these subjects the vertical movements of the larynx, observed externally, were recorded on 16 mm cinematographic film at 64 frames per sec. The onset of a laryngeal behavior was signaled by the subject by pressing an electrical contact which actuated one of the polygraph traces and also lit a synchronizing light in the field of view of the motion picture camera.

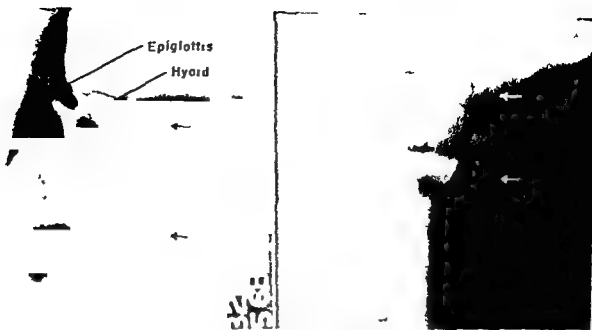


Fig. 1 Lateral radiographs of neck: left, end-expiration, right, vocalizing "ah" at 256 Hz. The upper arrow points to the hyoid bone; the lower arrow to the lower border

of thyroid cartilage. Note ascent of larynx toward hyoid bone with this vocalization.

RESULTS

1. Phonatory cycle

(a) *Pre-epiglottic spring* Laryngeal changes effected by phonating "ah" at 256 Hz are illustrated by Fig. 1, which presents lateral radiographs of the larynx in end-expiration (left) and during the phonation (right). With phona-

tion "ah" at 256 Hz the larynx became displaced toward the hyoid bone. The pre-epiglottic body was distorted and the hyo-epiglottic ligament became elongated, as shown by the increased distance between the anterior border of the hyoid bone and the epiglottis. The increase in the anteroposterior dimensions of the pre-epiglottic

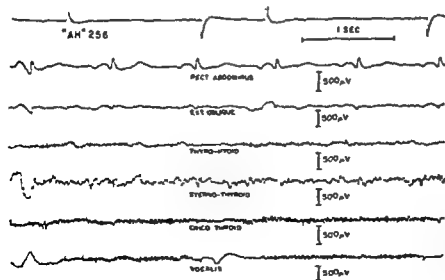


Fig. 2 Electromyograms during two successive vocalizations of "ah" at 256 Hz. The periods of phonation are indicated by the increased pen excursions in the maximum phonation record (lowermost trace).

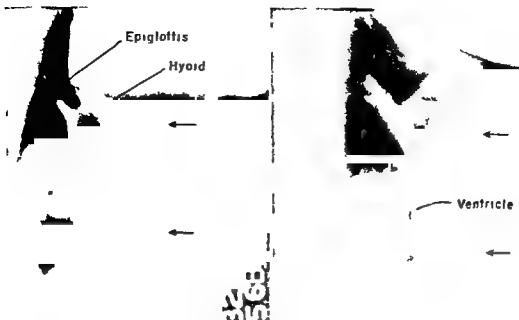


Fig 3 Lateral radiographs of neck: left, end-expiration; right, vocalizing "oooh" (u) at 256 Hz. Note descent of larynx away from hyoid bone with this vocalization

body probably also stressed the thyro-epiglottic ligament. Electromyograms from the same subject during successive "ah" phonations are presented in Fig 2. The periods of phonation are timed by the microphone (bottom trace). Increased activity in the thyrohyoid muscle

accompanied phonation but ceased with the end of phonation. Activity in the sternothyroid did not increase at the end of phonation, although cinematography of the neck showed that the larynx descended to its prephonatory station at this time. The stretched hyo-epiglottic ligament

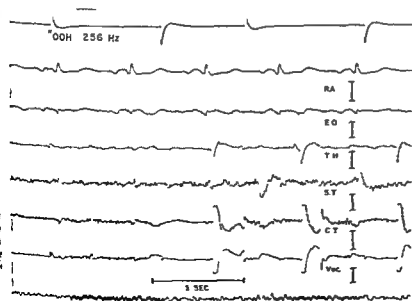


Fig 4 Electromyograms during two successive vocalizations of "oooh" at 256 Hz.



Fig. 3. Left: successive frames from motion picture filmed at 16 f.p.s., showing adduction of vocal folds at onset of low pitch phonation (Bell Telephone). Vibration of the folds, marked by blurring, is present in the last frame. Compression of the corniculates against each other occurs in the course of adduction. Also note concave margin

of the vocal folds developed during the adduction. Right: two frames from a motion picture of phonatory adduction, 11/16 second apart (von Leden & Moore, 1957). Note the concavity developed at the ventricular margin of the vestibular folds.

suggested by Fig. 1 and the absence of increased sternothyroid activity during the phase of restitution in Fig. 2 indicate that a strong element of elastic recoil contributed to the descent, including recoil of the hyo-epiglottic and thyro-epiglottic ligaments.

Figs. 3 and 4 show the corresponding events during phonation "ooh" (Q) at 256 Hz. Relative to end-expiration the phonating larynx (Fig. 3 right) was displaced downward, the pre-epiglottic body became elongated and, by inference, torn of its limiting ligaments, the hyo-epiglottic

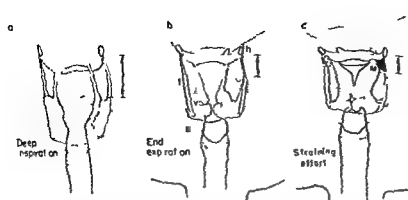


Fig 6 Tracings of anteroposterior tomograms of neck: left, deep inspiration; middle, end-expiration; right, straining effort. The pictures were aligned at the level of the hyoid bone (*h*). The outline of the thyroid cartilage lamina (*l*) is seen lateral to the vocal and vestibular folds.

The divergence upward of the laminae is most marked in deep inspiration and least marked during effort closure. The tracings were made by a medical artist instructed by an observer, both of whom were naive with respect to the questions at issue.

ligament and the thyrohyoid membrane, became stretched. Electromyography (Fig 4) recorded little or no increase in the thyrohyoid and sternothyroid activity during the period of vocalization and no increase in activity immediately following when the descended larynx was seen to return upward. The presence of elongated ligaments during the period of sound production and the absence of electrical activity in the extrinsic muscles during the period of restitution point to participation of passive elastic recoil in returning the larynx to its prephonatory station.

(b) *Corniculate spring*. Successive frames from the Bell Telephone motion picture (16 frames per sec; Fig 5, left, from above down) depict phonatory closure of the glottis. As adduction of the vocal folds proceeds, contact between the corniculate tubercles is observed to precede contact between the vocal processes. By the time the vocal processes meet, the elastic corniculate cartilages appear to overlap or are compressed against each other and form a single eminence. On cinematographic projection compression was clearly evident. When the adductor action ceased, recoil of the stressed corniculates would automatically initiate restoration of the respiratory passage at the glottis.

(c) *Conus elasticus spring*. The concavity some-

times developed by the free edge of the vocal folds during adduction (Fig 5 left) is a sign of tension tending to pull the vocal folds laterally. Tension in the subjacent conus elasticus presumably contributes to this phenomenon. Such tension may enter into play as an abductor force when the adductor contraction ceases. The concavity sometimes seen in the ventricular margin of the vestibular fold under similar conditions (Fig 5 right) also bespeaks lateral

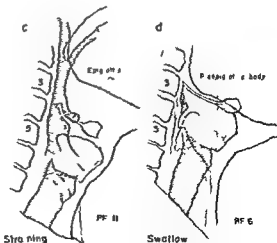


Fig 7 Tracings from lateral radiographs of neck during straining effort closure of larynx (left) and swallowing closure (right).

STRAINING

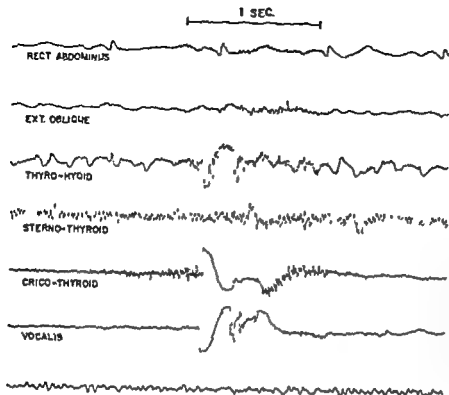


Fig 8 Electromyograms of straining effort closure of larynx. Onset of the effort is signaled by activity in the abdominal muscles (upper 2 traces). The large displacements in the 3rd, 4th and 5th traces are artefacts due to movement of the neck caused by ascent of larynx.

tension. Such tension will abduct the vestibular folds when arytenoid adduction relaxes.

(d) *Membrana quadrangularis spring*. Vocalization-associated retraction of the vestibular fold away from the vocal folds is detectable in Fig 3, where it is manifested by the vertical widening of the ventricle. The widening can be interpreted in part as preventing damping of the phonating fold, and is apparently due to tension in the membrana quadrangularis created by descent of the larynx and perhaps by contraction of the ary-epiglottic muscle (Fink, 1962). A similar effect has been demonstrated with laryngeal descent during deep inspiration (Fink et al., 1956). In either case, passive elastic recoil of the stretched membrana quadrangularis will assist restoration of the pre-existing relationship.

2. Straining effort closure cycle

(a) *Pre-epiglottic spring*. In Fig 6 tracings from anteroposterior tomograms of the larynx in inspiration, end-expiration, and effort closure

are aligned at the hyoid bone. The characteristic ascent of the larynx with effort closure is apparent from the position of the thyroid cartilage relative to the hyoid bone. In Fig 7 (left) tracing of the lateral X-ray view of the larynx during straining is presented. The increased hyoid-epiglottis distance that is associated with decreased thyroid-hyoid distance is apparent. This indicates that the hyo-epiglottic ligament was stretched during laryngeal ascent, and the recoil of the ligament will assist the return descent to the open configuration of the larynx.

Electromyographically (Fig 8) the period of effort and of elevation of the larynx is indicated by the presence of activity in the abdominal muscles. This activity is approximately co-extensive with the period of thyrohyoid activity. During restitution of the larynx at the end of the abdominal effort, there was little sign of electrical activity in the strap muscles. Again, a mainly passive recovery phase can be inferred.

The vestibular folds are known to be adducted

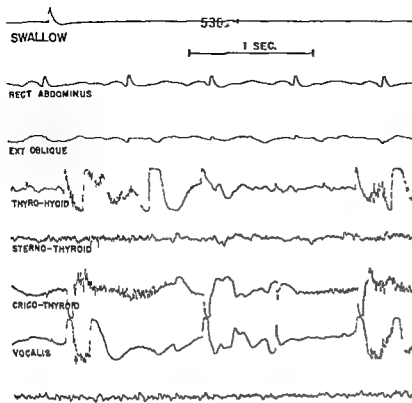


Fig 9 Electromyograms of swallowing closure of larynx. The signal in the uppermost trace was triggered by the subject's hand at the onset of swallowing. The large displacements in the 4th, 6th and 7th traces are movement artefacts. The thyrohyoid trace shows electrical activation of the muscle superimposed on a movement artefact at the onset of swallowing and a movement artefact without muscle activity at the end of the swallow. The beginning of a second swallowing act is seen on the right.

during effort closure (Fig 6) (Lindsay, 1942), necessarily straining the elastic connective tissue external to them. Restitutory retraction of the vestibular fold and cuneiform cartilage may be attributed partly to the recoil of elastic fibers in the connective tissue between the thyroarytenoides and the thyroid cartilage.

(b) *Thyroid cartilage spring* Evidence of spring action by the thyroid cartilage is detectable in Fig 6. The laminae of the cartilage are fixed below by the articulation of the inferior cornu with the cricoid cartilage but are free to bend inward and outward above. The laminae diverge from below up and the angle of divergence can vary if the lamina is pliable. In the subject of Fig 6 calcification of the thyroid cartilage was inconspicuous and the angle of divergence of the laminae did change with the functional state of the larynx. In the tracings of Fig 6 the angle of divergence is greatest in deep inspiration (left) and smallest in the closed larynx (right).

These appearances can be explained if the sternothyroids, in pulling the laminae of the thyroid cartilage downward in deep inspiration, also tend to pull them apart, and if, during effort closure the thyrohyoids pulling the laminae upward will also tend to pull them together. In either case, the strained cartilage will resume its equilibrium end-expiration configuration through its own elasticity. The spring action of the thyroid cartilage has been more fully discussed elsewhere (Fink 1973).

3 Swallowing closure cycle

(a) *Pre epiglottic spring* The X-ray tracing (Fig 7, right) shows that elevation of the larynx in swallowing closure was associated with extreme deformation of the pre-epiglottic body and great elongation of the hyo-epiglottic ligament. It is clear that return to the end-expiration configuration (Fig 1, left) will be associated with passive recoil of the structures stressed in ascent. The corresponding elevation

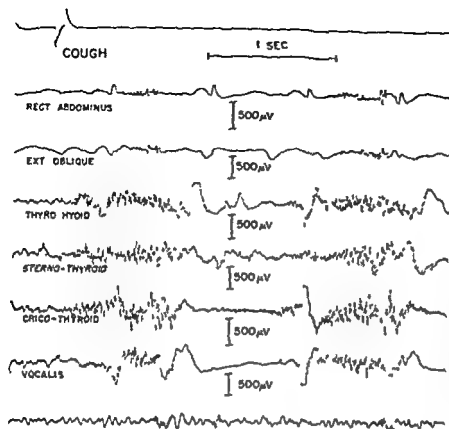


Fig 10 Electromyograms of coughing. Note (a) activity in the rectus abdominus, indicating the period of effort and laryngeal ascent, (b) increased blackness in the microphone trace at the bottom of the figure indicating the expiratory blast of air. Cinematography showed that laryngeal descent accompanied (b)

(Fig 9) show increased electrical activity in the thyrohyoid and sternothyroid muscles at the start of the swallow, ending about 0.7 sec later. These potentials were followed in the thyrohyoid trace by a movement artefact which cinematography identified as synchronous with the return descent phase of the laryngeal movement. The decreased activity in the thyrohyoid and sternothyroid muscles at this time indicates that as regards these muscles the restitutory descent was passive.

(b) *Epiglottic cartilage spring* Normally erect, the epiglottis usually folds over backward in the act of swallowing (Fig 7, right) apparently propelled by pressure from the tongue and pharyngeal muscles. Buckling of the cartilage may be facilitated by the above noted forward pull at the line of attachment of the hyo-epiglottic ligament during closure of the larynx. The elasticity of the epiglottic cartilage makes it snap back passively to its equilibrium configuration at the end of the act.

4 Cough cycle

Cinefluorographic and electromyographic (Fig. 10) examination of two subjects during deliberate coughing indicated that the mechanism of laryngeal closure during a cough resembled that of effort closure. However, the reopening phase, as timed by the microphone trace in Fig 10, differed inasmuch as activity in both the thyrohyoid and sternothyroid muscles was sustained at a somewhat decreased level for over 0.1 sec during this phase. It appears that in cough the restitutory descent of the larynx is actively assisted.

DISCUSSION

The data permit only tentative conclusions concerning the role of passive recoil, because extrinsic muscles other than those examined could have been involved. However, most of these, the stylohyoid, the digastric and the mylohyoid are designated as elevators of the

larynx and are of lesser significance to the question of restitutory descent. The omohyoid was the only depressor of the larynx not examined. The sternohyoid was studied in one subject and its responses were similar to those of the sternothyroid.

My radiographic findings corroborate those of Ardran et al (1953), and my electromyographic observations concerning the thyrohyoideus in swallowing are consistent with those of Doty & Bosma (1956), in dog, cat and monkey. In these animals the thyrohyoideus activity terminated very shortly after that of the lead group of muscles of deglutition. However, information expressly related to the laryngeal restitution phase on swallowing does not seem to be available (Kawasaki et al, 1964, Doty, 1968). In general, discussion of the "release" of the so-called sphincter mechanism of the larynx has been remarkably scarce (Pressman & Kelemen, 1955, Saunders, 1964 Plate IV).

The profusion of elastic tissue in and around the larynx is common knowledge (Katsumata, 1963) but its presence has not been correlated with function as clearly as has the elastic tissue in the lungs. Mink (1916) many years ago ascribed the return excursion of the larynx after inspiratory descent to its elastic connections, but the role of this tissue in other laryngeal activities has received little attention. Remmers & Gautier (1972) considered passive glottic reopening a possibility in feline purring.

Summarizing the available evidence, one may regard the station of the laryngeal passage at end-expiration as intermediate between the wide-open, caudally displaced larynx of deep inspiration and the closed, cranially displaced larynx of effort closure and swallowing closure. The intermediate partly open larynx of eupneic end-expiration represents in part an equilibrium between opposing elastic forces respectively pulling the larynx caudad and cranial. There is evidence that, in addition, tonic activity of "intrinsic" laryngeal muscles operates to maintain the functional end-expiratory position of the arytenoids (Fink et al, 1956, Kotby & Haugen, 1970).

A limited analogy thus exists with the respiratory excursions of the chest, where costal and pulmonary parenchymal "springs" are in equilibrium at eupneic end-expiration (Comroe et al, 1962). Both in chest and larynx, respiratory displacements from the equilibrium are active but can be restored by passive elastic recoil. In the human larynx this organization also appears to be the foundation for other behavior cycles, including phonation, effort closure, and swallowing closure. As regards the intrinsic muscles, their mode of action at the glottis during phonation and respiration has long been controversial and may deserve re-examining in the light of the above postulated synergism between active and passive forces.

Elastic restitution, being automatic, economizes neural energy that would otherwise be expended on timing the contraction of antagonist muscles. It may possibly effect a small economy in muscular energy as well, since elastic restitution utilizes energy "stored" in structures strained during the active, agonist phase. The substitution in man of a hyo-epiglottic ligament for the hyo-epiglottic muscle of lower primates and non primates illustrates the point. It is perhaps also significant that a median subhyoid air sac occurs in some lower primates in a position anatomically corresponding to that of the pre-epiglottic body of the hominoids. When closed, such a sac would constitute an excellent spring.

ZUSAMMENFASSUNG

Kehlkopfbewegungen nehmen normalerweise aktiv an beiden Phasen der Funktionszyklen teil, wie z. B. Phonation, Räuspern und Schlucken. Aufgrund röntgenologischer, elektromyographischer und kinematographischer Untersuchungen wird angenommen, dass diese Zyklen mit einer Phase aktiver Muskelkontraktion beginnen und mit einer Phase teilweise passiver elastischer Rückformung enden. Diese elastische Rückformung ist möglicherweise ein allgemein bedeutender Faktor beim organisierten Ablauf der Kehlkopfbewegungen.

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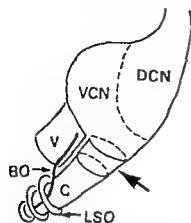


Fig 1 The area sectioned from the cochlear nerve VCN = Ventral cochlear nucleus DCN = Dorsal cochlear nucleus V = Vestibular nerve C = Cochlear nerve BO = Bundle of Oort LSO = Lamina spiralis ossea Arrow points to sectioned area

cats. We were also interested in knowing whether the unimodality of the fibres described by Rasmussen & Gacek (1961) would appear in electron microscopic sections.

MATERIAL AND METHODS

Fixation

Three cats weighing respectively 2, 2.5 and 3 kg were anesthetized with Nembutal administered peritoneally, 0.5 ml per kilo bodyweight. Destruction of the left cochlea was then performed, and after 3 days they were perfused via the abdominal aorta. The perfusion liquid consisted of 250 ml NaCl (pH 7.45), 500 ml paraformaldehyde 4% in Millonig's buffer (pH 7.45) and 1000 ml glutaraldehyde 2.5% in Millonig's buffer (pH 7.45).

5 ml 1% calcium chloride per litre was added to each liquid. 0.5 ml Heparin was injected into the inferior caval vein before perfusion.

Preparation

The brain and medulla were freed from the bony surroundings, and left overnight in glutaraldehyde. The next day the right cochlear nerve was isolated proximal to the bundle of Oort in order to exclude the efferent fibres (Fig 1). A section of the nerve, approx. 1–1 mm was cut

out distal to the point where it enters the cochlear nuclei. The tissue pieces were then washed in Millonig's buffer 3×10 min and left for 2 hours in osmium 1% added to Millonig's buffer. The blocks were then dehydrated in acetone 50%, 70%, 85% and 96%, 10 min in each liquid, and thereafter in 100% for 25 minutes. Then they were transferred to 100% acetone and Epon 812 for 15 min, and left overnight in Epon and DMP.

The blocks were embedded in capsules of acetate foil and left for 12 hours in 100°C. Sections 400 Å were then cut randomly on an LKB Ultratome. The sections were taken from the periphery as well as from the centre, and in different places, square to the longitudinal course of the nerve. They were placed on grids and stained with lead citrate.

Photographic procedure

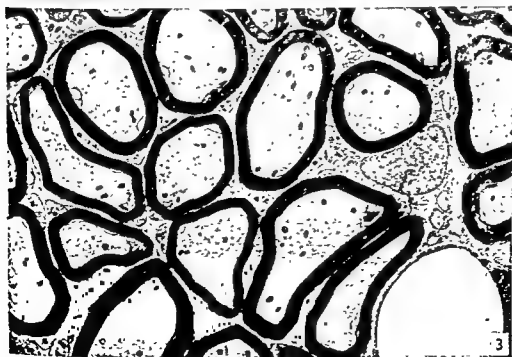
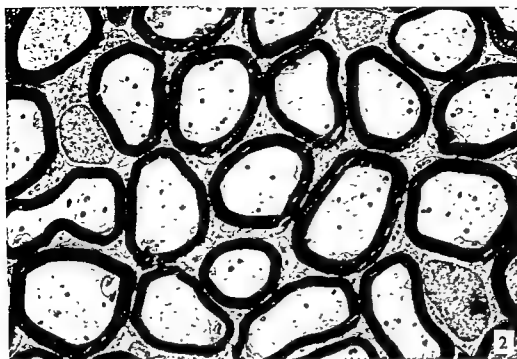
A Siemens Elmiskop was used for taking the micrographs. Primary magnification was $3000 \times$, and later the photographs were enlarged $\times 3$. Since the fibres, with exception of the outer ones, are spirally arranged through the nerve, it is impossible to present only square-cut fibres in a micrograph. This difficulty was partly overcome by taking a sufficient number of pictures in different planes and by measuring a multitude of fibres. An Aristo planimeter (model 1130) was employed to measure the square areas of (a) the axon,¹ (b) the axon + the myelin sheath (Figs 2 and 3).

Planimetry was performed in 1266 different areas, and by subtraction the area of the myelin sheath could also be calculated.

RESULTS

The results are shown in Figs 4–6. The figures show the areas measured in μm^2 of axon, axon + the myelin sheath, and myelin sheath alone, in the cochlear nerves of the 3 cats. Some difference is observed between the three cases, but

¹ The expression axon in this communication used for the area inside the myelin sheath, i.e. the area limited by the axolemma.



Figs 2-3. The axons surrounded by their myelin sheaths

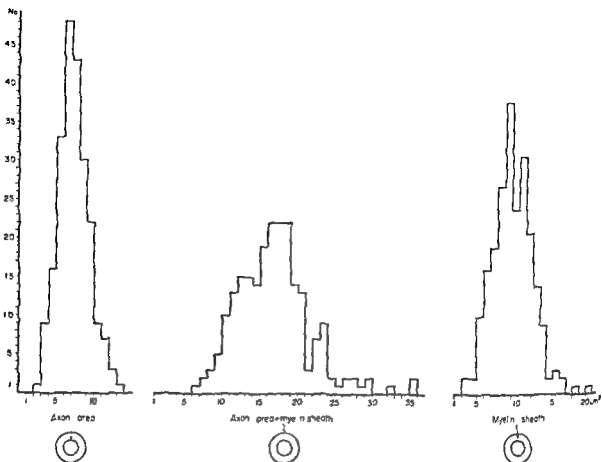


Fig 4 Cat 1. Each column represents 222 planimetric measurements (in all, 666)

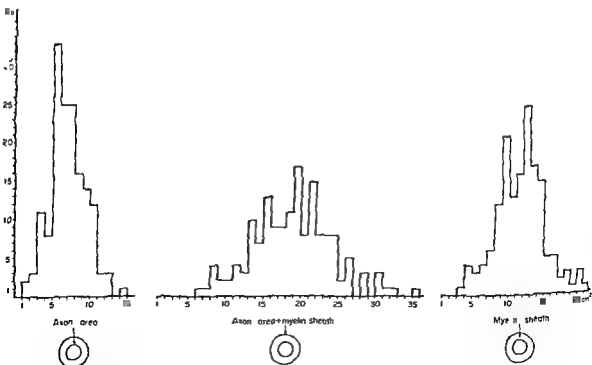


Fig 5 Cat 2. Each column represents 156 planimetric measurements (in all, 468)

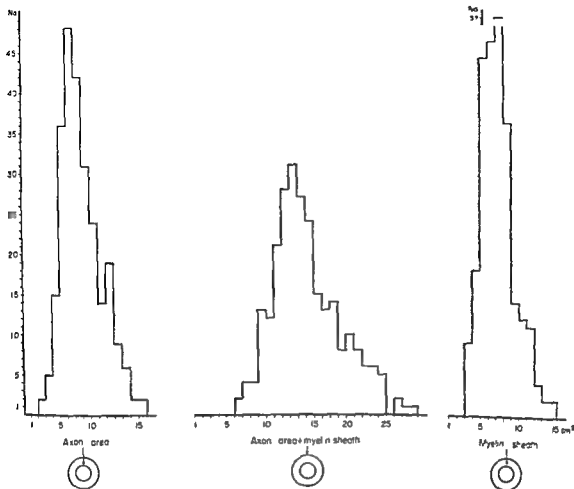


Fig 11 Cat 3 Each column represents 255 planimetric measurements (in all, 765)

none greater than is explicable by individual variations

Thereafter, the true diameters were calculated (by computing the area with πr^2) and likewise the areas, taking into account the enlargement. The two diameters and areas (maximum and minimum) are shown in Table I. In this table, however, the extreme values are omitted. The explanation for this procedure is that some of the pictures will show fibres which are obliquely cut, due to their spiral course in the nerve. These, which are in the majority, will give an area greater than the average. Other fibres will have shrunk during preparation, and their circumference will be curved inwards, thus giving a smaller area.

Table II shows that if 23% of the greater and 9% of the smaller areas are omitted, 68% of the fibres (axon + myelin sheath) varies between 4 and 6 μm .

Table I Minimum and maximum diameters and areas (in μm)

	Axon + myelin sheath	Axon	Myelin sheath
Minimum—maximum diameter, μm	4–6	2–4	1–2
Average diameter, μm	5	3	1.5
Minimum—maximum area, μm^2	14 25	4 15	6 19
Average area, μm^2	19	9	12

Table II *Distribution of fibre diameters, in percentages*

	Axon + myelin sheath	Axon	Myelin sheath
Percent fibres within minimum and maximum calculated area	68% (4-6 μ m)	92% (2-4 μ m)	65% (1-2 μ m)
Percent above calculation	23%	6%	18%
Percent below calculation	9%	2%	17%

Of course the average figures might be given with a greater variation, but Table II clearly shows that the majority of calculations (68-92-65%) do not differ in diameter more than 2 μ m, whether the axon and the myelin sheath, the axon alone, or the myelin sheath alone, is taken into account

DISCUSSION

Various, although to a large extent coinciding dimensions, have been published concerning the calibre of the VIII nerve fibres (Kolliker, 1852 1-2.5 μ m, Kolmer, 1927 3-5 μ m Engstrom & Rexed, 1940 1-9 μ m, and Rasmussen, 1940 3-10 μ m). All the measured fibres were in man. Rasmussen & Gacek (1961) found the calibre in cat to vary between 1 and 8 μ m. A variation between 1 and 10 μ m would thus cover all the previous findings. These, however, were performed with a light microscope and oil immersion lenses, whereas the present authors have applied the electron-microscope. The large magnification used by us gives more accurate results and the planimeter is a very reliable instrument. Its main error is only 0.9% (Hall, 1964).

Taking into consideration also the multitude of areas measured (1 266), and the large magnification (9 000 linear), the methodological errors should be practically negligible when the numbers are reduced to the true size (in μ m).

The unimodal distribution of the fibres,

described by Rasmussen & Gacek (1961) was also noted in the present investigation. The distribution is seen from the figures 4-6. If fibres to the inner and outer hair cells had different calibres, distinct double-humped curves would have been seen. This is not the case. Further more, the results are, when diameters are considered, in accordance with those of Engstrom & Rexed (1940) and Rasmussen (1940).

ACKNOWLEDGMENT

The authors are indebted to Professor F Walberg for valuable advice and criticism and to the photographer B Bratil, for excellent technical assistance and planimetry.

ZUSAMMENFASSUNG

Die Verfasser haben durch Planimetrie auf elektron mikroskopischen Schnitten den Diameter der Faser des Nervus Cochlearis gemessen.

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BEHAVIORAL AUDITORY FUNCTION AFTER TRANSECTION OF CROSSED OLIVO COCHLEAR BUNDLE IN THE CAT

II Conditioned Visual Performance with Intense White Noise

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Abstract Feline subjects were trained to respond to a visual signal in the presence of distracting background white noise. The fixed 'performance ratio' was measured by varying the intensity of the background white noise. The experimental results indicated that the elimination of the crossed olivo-cochlear bundle resulted in an increase in white noise distraction of the light signal detection task in the cat. Statistical analysis confirmed a significant difference between the experimental and sham groups. The electron microscopic and neurohistological investigations confirmed the disappearance of the efferent nerve endings in the cochlea and that proper midline olivo-cochlear bundle sections had been made. Probably one way in which the crossed olivo-cochlear bundle operates to inhibit acoustic processing is by the activation from a sensory system of a different modality.

Neurophysiological studies of the olivo-cochlear bundle (OCB) after electrical stimulation or interruption of it have been conducted by many investigators. After electrical stimulation of the efferent fibers, many investigators including Galambos (1956, 1960) and Fex (1962) considered the role of this system to be inhibitory in nature, or at least in part.

A study by Pfalz in 1969 on the other hand described absence of function of the crossed OCB under physiological conditions in the guinea pig. In our study (Igarashi et al., 1972)

after transection of crossed OCB, the pure tone threshold (250-14 000 Hz) was not altered, and neither was any change found in the perceptual signal/noise ratio at the levels of 30, 50, and 70 dB above the subject's pure tone threshold. Neuro-histological investigation of the brain stem confirmed that proper surgical lesions had been made in the brain stem. Also, there had been clear degeneration of the efferent nerve endings around the hair cells, especially in the basal coils (electron microscopic investigation) Irvine & Webster (1972), measuring cochlear microphonic and auditory nerve action potentials in unanesthetized cats, concluded that the OCB does not function as a peripheral gating mechanism in the auditory system.

Guzman-Flores & Alcaraz's experiments (1963) showed that lesion of the OCB abolishes the attenuation of cochlear evoked potentials during attentive behavior to a visual stimulus, which suggests that the auditory and visual systems are connected by the OCB.

The present study was conducted in order to examine whether the crossed OCB might be activated by a non-auditory stimulus (i.e., a visual signal) during exposure to intense background white noise. The hypothesis of the investigation is that the OCB plays the role of gating an irrelevant auditory signal when a more significant stimulus is presented.

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Subjects

Six screened cats were used for the present study. They were clean healthy young adult cats (with no preference as to their sex) with no otological disorders. Intact tympanic membranes were revealed by the otoscopic investigation and also a clean middle ear was confirmed at the time of specimen removal for electron microscopy.

Preoperative procedure

Subjects were screened by an avoidance conditioning method using a cat rotating cage in a sound proof chamber (IAC 1202). Subjects were required to turn the cage within 5 sec after the presentation of the flashing light signal. A light box was made of plexiglass covered with black paper. The size was 7 cu inch. A small circular hole (1/2 in diameter) in the front was the only opening of this box not covered with black paper. The box was suspended about 30 cm from the subject (in the cage) at his head level. The speaker which delivered white noise was installed at a higher level so that the box would not interfere with the noise presentation. White noise (15 dB above 0.0002 dyne/cm²) was constantly used to mask a faint click produced by the light. The ambient light intensity of the testing chamber approximated the level of light outside the chamber so that light adaptation was not a factor.

The subjects were further trained to rotate the cage while the light signal was on to a 90% correct response criterion. The training was continued for one week. Then the intensity of the light was gradually (step wise) decreased to reach the subjects' positive response thresholds (dimmed light detection threshold). This particular procedure required 2 to 3 weeks. Thereafter the light intensity was increased up to the 80% correct response level and stabilized at that level which was specific for each subject. Background white noise (500–15 000 Hz) was then introduced and was gradually increased in 5 dB steps until the intensity of white noise was reached at which the subjects responded to the light at only a 50% correct performance ratio

(distraction level). At least six preoperative measurements were obtained.

Surgery

Animals were anesthetized with sodium pentobarbital (30–40 mg/kg body weight). An occipital midline incision was made to expose the posterior portions of the cerebellum and brain stem. The cerebellum was gently displaced upward by a malleable metal retractor. The crossed OCB was cut in the floor of the fourth ventricle with a fine pick. The incision was extended about 2–3 mm both rostrally and caudally from the 11 mm point anterior to the obex. Three cats were assigned to the transection of the crossed OCB while the other three were randomly used for the sham control operation exposing the region retracting the cerebellum but not sectioning the crossed OCB.

Postoperative procedure

A two week period was allowed for each subject to recuperate from surgery. Immediately after this period the dimmed flashing light detection threshold for each subject (experimental and sham) was remeasured. After the animals were able to respond at a mean rate of 80% or greater for four trial days at the initial 80% correct performance rate intensity the background white noise was introduced. The distraction level was measured according to a method identical to that used preoperatively.

After obtaining sufficient postoperative data all subjects were sacrificed for the purpose of morphological investigation. After local perfusion with 2% osmium tetroxide solution (Millonig) cochlear specimens were removed and processed according to the standard procedure. Specimens were embedded in Epon and ultrathin sections were cut with a Porter Blum ultramicrotome. Ultrathin sections were stained with uranyl acetate and lead hydroxide and were studied under a JEM 7 electron microscope. Sections obtained from at least five different areas (from basal and middle turns) of each cochlea were studied.

The surgical area of the brain stem was pre-

pared separately. The serial cross sections of the brain stem were studied after cresylecht violet, galloyanune or methylene-blue staining. By this procedure, the depth and extent of the surgical lesions were confirmed.

RESULTS

After the two week recuperation period, the dimmed flashing light detection threshold for each subject (both in experimental and in sham groups) was remeasured and no change in it was observed. The light intensity level of postoperative 80% correct response for each subject also remained the same. The sham controls required a mean of 6.0 days to reach the criterion (4 days, 80% correct response) while the experimentals, a mean of 9.6 days.

With background white noise introduction, different results were obtained between the experimental and the sham subjects. The animals which belonged to the experimental group postoperatively required less intense white noise to distract them from the 80% correct response level when compared with the preoperative state. At least 72 dB noise (re 0.0002 dyne/cm²) was required to demonstrate noticeably more distraction in experimental cats. All subjects in the sham operation group required slightly higher noise intensity to distract them from the 80% correct response level. The improvement

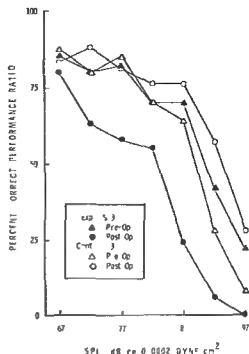


Fig. 1 This graph displays the comparison of the distracting noise level between the averages of experimentals and shams.

in the performance of the sham animals is considered to be due to a training effect.

Table I summarizes the pre- and postoperative results of functional testings. Statistical analysis by *t* test confirmed a significant difference between the experimental and sham groups ($t = 5.30$, $p < 0.005$). Therefore, it is suggested from the results that the elimination of the crossed OCB resulted in an increase of white noise distraction of the dimmed flashing light signal detection task in cats. Figure 1 graphically displays that less intense noise distracts visual performance in experimentals postoperatively.

Electron microscopic investigations confirmed the disappearance of the efferent nerve endings around the hair cells, especially the basal coils of the experimental subjects (Fig. 2). Also, the surgical lesions in the brain stems were neurohistologically confirmed to have been properly made to interrupt the crossed OCB. The midline sections extended about 2.0 mm (both rostrally and caudally) beyond the edges of the facial colliculi (Fig. 3).

Table I This table demonstrates the preoperative and postoperative distracting white noise level of the visual task.

The statistically significant difference was found in the required noise level between the experimental (X) and sham (S) groups. See text.

Subject	Noise level (dB re 0.0002 dyne/cm ²)		
	Pre-Op	Post-Op	Difference
XA	95.5	84.8	10.7
XB	89.0	84.8	- 4.2
XC	90.0	78.5	11.5
SA	88.0	90.8	2.8
SB	92.0	96.5	4.5
SC	89.0	92.8	3.8



Fig 2 The electronmicrograph demonstrates the disappearance of efferent nerve endings at the inferior portion of the outer hair cells (arrows). A, afferent nerve endings. This specimen was obtained from the upper basal turn $\times 7\,000$

DISCUSSION

In 1971 Borg measured the response of the acoustic middle ear reflexes in unanesthetized and unrestrained rabbits. Chronic lesions were made to interrupt the crossed OCB. Only inhibitory influences were found. It was suggested that the inhibitory influences observed may be due mainly to tonic spontaneous activity from the efferent fibers. In an experiment of the cross modality between middle ear muscles and the visual system, Simmons et al (1959) trained

feline subjects to expect a loud noise when a light appeared. Middle ear muscles were cut on one side. Evidence of successful training was given by demonstrating a conditioned response of the middle ear muscles on the uncut side when the light alone was presented. The experimental results demonstrated that middle ear muscle excitation can be altered by a visual stimulus.

Inasmuch as in the present study the middle ear muscles were not cut, it is not known whether the results obtained are due to the inhibitory

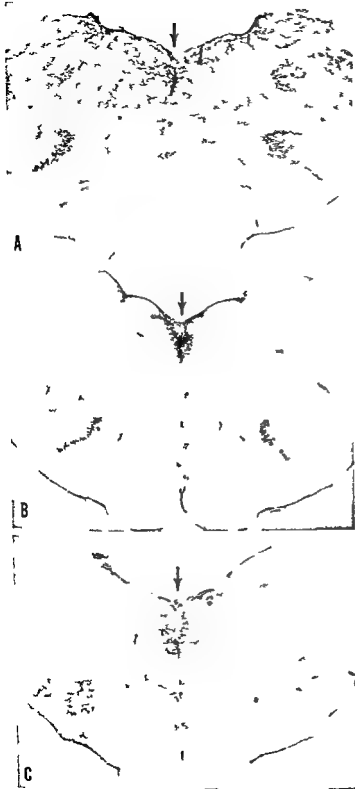


Fig 3 Three transverse sections of the brainstem demonstrate the rostral-caudal distribution of midline cutaneous afferents. A about 2 mm rostral to the level of the facial genu B at the level of the facial genu C about 2 mm caudal to the level of the facial genu. Cresyl-euchrome, $\times 100$.

mechanism at the peripheral end organ level, or to a functional inhibition at the level of the middle ear muscles. It is noteworthy, however, that in our previous study (Igarashi et al., 1972) with white noise of comparable intensity, no effect was noted after crossed OCB transection upon increased white noise masking. In addition, the existence of olivary complex-cochlear nucleus connections cannot be neglected. In any event, however, it was observed that the midline lesion has some effect when a subject responds to a visual stimulus under an intense background white noise, even though the lesion may affect in an unspecific way in animals' behavioral responses.

Harrison & Irving (1966), after studying the monkey, bat, dolphin, guinea pig, rat, mouse, and hedgehog, described how the medial superior olive appeared to be a part of the auditory system which was in some way related to vision. The medial superior olive was well developed in all diurnal animals and in nocturnal animals with good vision such as cats, and in animals with well developed eyes. The medial superior olive was probably not concerned with auditory localization in the psychophysical sense, but was probably concerned with the movement of the

and eyes in the direction of the sound in

Thus, this area may be a contact between auditory and visual systems.

In addition to subcortical connections, connections may exist between visual system and the insular temporal cortex which is suspected as an auditory (integratory) cortex (Desmedt, 1960). In regard to the corticofugal connections, according to Rasmussen (1964) two cortical fiber connections are the corticogeniculate fibers and the fibers to the nuclei of the brachium and the inferior colliculus. Some fibers from the nucleus of the inferior colliculus enter the superior olivary regions, however the functional significance of these fibers is not known.

In 1959 Hubel and others found that while they were recording unit responses from the auditory cortex in unrestrained and unanesthetized cats, a population of cells appeared to be sensitive only when the subject paid attention

to the tone source. This experimental result very clearly indicated the importance of the functional investigation in unanesthetized subjects, and also suggested that paying attention can evoke or at least assist auditory neural function within a psychological unit.

It is known that in higher mammals (cats) the OCB has about 500 fibers which in turn supply 40 000 efferent nerve endings (Spoendlin, 1966). As such it is suggested that the OCB function, if any, is probably relatively general for the inhibition of auditory input. Probably one way in which the OCB operates to inhibit acoustic processing is by the activation from a sensory system of a different modality.

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ZUSAMMENFASSUNG

Versuchskatzen wurden trainiert auf sichtbare Signale zu reagieren während im Hintergrund ablenkender, weisser Lärm zu hören ist. Das bestimmte Verhältnis ihres Verhaltens wurde durch die Veränderung der Intensität des weissen Lärms im Hintergrund gemessen. Die Versuchsergebnisse bewiesen, dass die Katze ohne das gekreuzte OCB (Olivio Cochlearis Bündel) durch den weissen Lärm mehr von ihrer Aufgabe abgelenkt wird, als durch Lichtsignale zu reagieren. Eine statistische Analyse durch 1 Versuch bezeugte einen bedeutenden Unterschied zwischen Versuchs- und Scheingruppen. Die elektronenmikroskopischen und neurohistologischen Versuche bestätigten das Verschwinden der austragenden Nervenfasern in der Cochlea und dass richtige, mittel lineare OCB-Sektionen gemacht wurden. Eine Möglichkeit in welcher das OCB so funktioniert, dass es den Hörprozess kontrolliert, besteht wahrscheinlich in der Aktivierung des OCB durch ein Empfindungssystem mittels eines anderen Verfahrens.

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THE RELEASE OF ACETYLCHOLINE (ACH) BY THE CROSSED OLIVO COCHLEAR BUNDLE (COCB)

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Abstract A method has been developed to detect acetylcholine like activity in the perilymph of guinea pigs. The acetylcholine like activity in the guinea pig perilymph is not significantly increased by moderate acoustical stimuli but it is significantly increased when the crossed olivo-cochlear bundle is tetanically stimulated. The criteria for identifying acetylcholine as the chemical mediator at the crossed olivo-cochlear nerve/hair cell junction are now largely fulfilled.

The possibility that the junctional transmission between the olivo-cochlear nerve endings and the cochlear hair cells is chemically mediated has been suggested by many authors (Gisselsson, 1960, Fex 1962, Amaro et al, 1966, Katsuki, 1966, Daigneault & Brown, 1966, Bobbin & Konishi, 1971). Of all the neurohumors said to operate at this junction, the candidacy of acetylcholine (ACh) has received the most experimental support. The strict localization of the enzyme, acetylcholinesterase (AChE), to the crossed olivo cochlear bundle (COCB) endings demonstrated by Churchill et al (1956) initiated the idea that ACh may be the transmitter at these endings. Since that time many other investigators have studied the histochemical localization of AChE to the cochlea using both light microscopy and electron microscopy. An excellent summary of these findings is given by Iurato et al, 1971. However acetylcholinesterase is a rather ubiquitous enzyme and its presence alone

is not sufficient proof that a particular junction is cholinergic in nature.

The identification of a neurohumor depends upon the substantiation of several criteria (Eccles, 1964). A typical list as given by both Werman (1966) and Phillis (1970) is as follows:

- 1) *Release* During stimulation, the candidate substance should be demonstrable in the extracellular fluids collected from the region of the activated synapse.
- 2) *Duplication* Exogenous application of the candidate substance to the post synaptic membrane should mimic the action of the synaptically released transmitter.
- 3) *Pharmacological* Pharmacological agents which interact with the putative transmitter at other biological sites should have analogous interactions at the synapse under consideration, depending upon the properties of the receptors.
- 4) *Termination* An inactivation system should be demonstrable.
- 5) *Synthesis* There should be present the necessary structural and enzymatic mechanisms for the manufacture and storage of the transmitter.

The criteria of duplication, termination and synthesis have been conclusively fulfilled for the acetylcholine like activity at the COCB hair cell synapse. That is, Bobbin & Konishi (1971) reported that ACh mimics COCB stimulation. Churchill et al (1956) demonstrated the presence of the terminating enzyme acetylcholinesterase, and Jasser & Guth (1973) demonstrated the presence of the ACh synthesizing enzyme choline

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acetyltransferase The criterion of pharmacological interactions is supported by some data and can be questioned by other data Apparently damaging to the fulfillment of the pharmacological criterion is the finding of Desmedt & Monaco (1960) that strychnine, which was not known to be an anticholinergic, blocks the effects of COCB stimulation The work of McKinstry & Koelle (1967) later showed that strychnine could inhibit the release of acetylcholine Thus the strychnine block may be supporting rather than detracting evidence Also damaging to the pharmacological criterion is the fact that Desmedt & Monaco (1961) were unable to block OCB effects with dihydro-B erythroidine Sohmer & Feinmesser (1963) could not measure changes in cochlear microphonics (CM) or gross eighth nerve action potentials (AP) by using atropine and physostigmine Tanaka & Katsuki (1966), using iontophoretic means, could not relate the effects of various cholinergic and anticholinergic agents to the olivo cochlear inhibition of AP caused by electrical stimulation of the COCB It is possible that route of drug administration, or technical difficulties could account for the above negative results or it is possible that the hair cells have a new or modified type of cholinergic receptor

However, the cholinergic hypothesis is gaining additional support as methods become more refined and drugs are applied directly into the cochlear fluids rather than systematically For example, Guth & Amaro (1969) were able to demonstrate a classic hemicholinium-3 effect on the activity of the COCB, Fex (1968) was able to block COCB activity with d-tubocurarine, Galley et al (1971) blocked the AP inhibition caused by COCB stimulation by applying gallamine triethiodide directly to the scala tympani, and Bobbin & Konishi (personal communication) were able to block COCB activity with a fairly large series of standard anticholinergic drugs Finally, the criterion of release which may be considered the keystone criterion, is supported only by the preliminary discussions of Fex (1968) and Whitfield (1967) in the September, 1967 Ciba Symposium on Hearing Mechanisms in

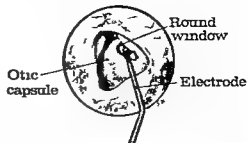


Fig 1 Post auricular approach to the round window showing placement of the recording electrode

Vertebrates Both mentioned pilot experiments which appeared to be positive for ACh but to this date neither of them has published the results of his experiments Therefore, the present work was done in order to determine whether or not the criterion of release could be substantiated Of all the criteria, this seems to be the most important to test and a positive result here could provide the strongest support for the cholinergic hypothesis

METHODS

Surgical technique

Young guinea pigs weighing between 150 and 200 grams, of either sex, are anesthetized with pentobarbital (25–30 mg/kg i.p.) At the start of surgery cannulas are introduced into the trachea, carotid artery and external jugular vein for purposes of artificial respiration, blood pressure measurements and drug administration, respectively Next, the structures around the ventral portion of the bulla are cleared away and the bulla is opened, exposing the middle ear and the otic capsule At this time both the stapedius and tensor tympani muscles are separated from their respective insertions After opening the bulla the animal is turned in a specially designed head holder and a post auricular approach is made to the round window A silver/silver chloride monitoring electrode is carefully placed on the round window membrane and cemented in place with zinc dental cement (Fig 1) This electrode is used to measure the response of the cochlea to an audible click or to a tone burst Next, the skull of the animal is opened from

THE RELEASE OF ACETYLCHOLINE (ACH) BY THE CROSSED OLIVO COCHLEAR BUNDLE (COCB)

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(Received March 20 1973)

Abstract A method has been developed to detect acetylcholine like activity in the perilymph of guinea pigs. The acetylcholine like activity in the guinea pig perilymph is not significantly increased by moderate acoustical stimuli but it is significantly increased when the crossed olivo cochlear bundle is tetanically stimulated. The criteria for identifying acetylcholine as the chemical mediator at the crossed olivo-cochlear nerve/hair cell junction are now largely fulfilled.

The possibility that the junctional transmission between the olivo-cochlear nerve endings and the cochlear hair cells is chemically mediated has been suggested by many authors (Gisselsson, 1960, Fex, 1962, Amaro et al., 1966, Katsuki, 1966, Daigneault & Brown, 1966, Bobbin & Konishi, 1971). Of all the neurohumors said to operate at this junction, the candidacy of acetylcholine (ACh) has received the most experimental support. The strict localization of the enzyme, acetylcholinesterase (AChE), to the crossed olivo cochlear bundle (COCB) endings demonstrated by Churchill et al. (1956) initiated the idea that ACh may be the transmitter at these endings. Since that time many other investigators have studied the histochemical localization of AChE to the cochlea using both light microscopy and electron microscopy. An excellent summary of these findings is given by Iurato et al., 1971. However, acetylcholinesterase is a rather ubiquitous enzyme and its presence alone

is not sufficient proof that a particular junction is cholinergic in nature.

The identification of a neurohumor depends upon the substantiation of several criteria (Eccles, 1964). A typical list as given by both Werman (1966) and Phillis (1970) is as follows:

- 1) *Release* During stimulation, the candidate substance should be demonstrable in the extracellular fluids collected from the region of the activated synapse.
- 2) *Duplication* Exogenous application of the candidate substance to the post synaptic membrane should mimic the action of the synaptically released transmitter.
- 3) *Pharmacological* Pharmacological agents which interact with the putative transmitter at other biological sites should have analogous interactions at the synapse under consideration.
- 4) *Termination* An inactivation system should be demonstrable.
- 5) *Synthesis* There should be present the necessary structural and enzymatic mechanisms for the manufacture and storage of the transmitter.

The criteria of duplication, termination and synthesis have been conclusively fulfilled for the acetylcholine like activity at the COCB hair cell synapse. That is, Bobbin & Konishi (1971) reported that ACh mimics COCB stimulation. Churchill et al. (1956) demonstrated the presence of the terminating enzyme acetylcholinesterase and Jasser & Guth (1973) demonstrated the presence of the ACh synthesizing enzyme choline

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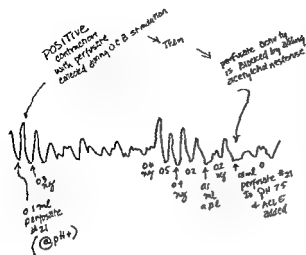


Fig 5 Bioassay recording Ileum contractile activity in 0.1 ml perfusate collected during COCB stimulation is reduced by incubation with acetylcholinesterase. One hundredth (0.01) μ l no 21 refers to perfusate samples. Numbers followed by ng refer to standard doses of acetylcholine in nanograms

pine to the Tyrodes bathing solution or by adding acetylcholinesterase to the active perfusate. The contractile activity of the perfusate samples should be blocked or significantly diminished after such manoeuvres if that activity was originally caused by acetylcholine. Fig 5 is a recording showing how an ileum contractile response elicited by collected perfusate is completely blocked the addition of acetylcholinesterase to the

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histology

Upon completion of the perfusion experiments in some of the animals, the temporal bones were prepared for histological examination by the surface preparation technique of Engstrom et al (1966). After some of the other perfusion experiments, the ears were prepared by the method of Guild (1921) as modified by Schuknecht (1953). The goal of these examinations was to determine if the perfusion technique had caused any observable cochlear damage.

Preliminary experiments

More than thirty preliminary experiments were performed. During these experiments, several of the parameters in the protocol were varied in

order to achieve the optimum results. For example, during preliminary experiments, the rate of application of the tetanic stimulation to the COCB was varied in order to test the possibility that the nerve could be fatigued. It was important to measure this possibility since the stimulation perfusions had to be carried on for more than 1 hour in order to collect enough perfusate. Fatigue was measured as a loss of AP inhibition. Optimal AP inhibition was usually equivalent to a 15 to 20 dB decrease in click intensity. If the COCB was stimulated at a rate of two tetanic stimulations per second, fatigue (2 to 6 dB of equivalent click intensity) could be seen within the first 5 sec and the inhibitory effects of COCB stimulation were completely dissipated within 10 to 15 sec. After a short period of rest (30 sec) the inhibition was restored. At tetanic stimulation rates of one each 2 sec, fatigue was seen after 35 min, however, tetanic stimulations of the COCB applied once each 5 sec did not produce any measurable fatigue for 1 hour or more and this was the rate used for most of the experiments.

In some of the early experiments, the calcium concentration in the artificial perilymph was 2.8 mM, but in most of the later experiments the calcium concentration was increased to 5.6 mM in an effort to obtain an increased yield of neurohumor. Higher calcium concentrations, up to 10 mM, were also considered, but when the artificial perilymph containing these concentrations was tested on the smooth muscle assay preparation it seemed to cause excessive spontaneous activity which prevented that particular assay preparation from being used again.

In another set of three experiments the cochlea was perfused with artificial perilymph containing known quantities of ACh. Bioassay of the collected perfusate showed that better than 90% of the ACh was recovered in each case. It was therefore concluded that the perfusion technique did not appear to cause any substantial losses in acetylcholine that might have been in the perilymph. Attempts were also made to store recovered perfusate in the freezer. Perfusate stored over-night lost up to 50% of its activity.

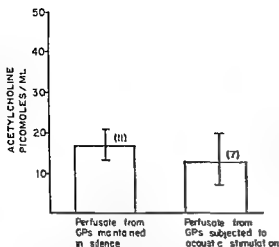


Fig 6 Background acetylcholine-like activity released into perilymph. Differences are not statistically significant. No electrical stimulation of COCB was used in either case. Click strength was 1 volt 80 msec pulse applied to TDH 39 earphone.

and perfusate stored for several days lost more than 50% of its activity. Storage in soft glassware also seemed to cause the perfusates to lose activity so that in all of the later experiments the perfusates were collected and stored only in hard glassware.

After the first preliminary experiments established that the collected perfusates exhibited ileum contractile activity, a protocol was initiated wherein all active perfusates were tested with either acetylcholinesterase or with atropine. In all cases tested, the contractile activity was completely blocked. In a few cases enough perfusate was collected so that both tests could be applied and again the ACh-like contractile activity was blocked. The concentration of atropine used did not block equivalent ileum contractile responses elicited by histamine, bradykinin and 5-hydroxytryptamine.

RESULTS

Data from 43 successful experiments are given in Figs 6, 7 and 8. Fig 6 compares the results from seven perfusion experiments during which the animals were subjected to free field clicks from the TDH 39 earphone and eleven other perfusion experiments during which the animals

were maintained in the relative silence of a sound dampened chamber. In no case was the COCB stimulated. The clicks were applied at the rate of two per second and were derived from the application of a 1 V, 80 msec square wave pulse to the earphone. All bioassay results are reported in terms of the picomoles of standard acetylcholine per milliliter which equivalently stimulate the ileum. An analysis of variance justified the use of the pooled *t* test which then demonstrated that no significant difference existed between the means of the two sets of data (perfusion during silence vs perfusion during click). It was therefore concluded that there was no significant difference between the amount of acetylcholine-like activity in the perfusates collected when the animals were maintained in silence or when the animals were subjected to acoustic clicks.

Fig 7 compares the results from twelve experiments in which control perfusions were

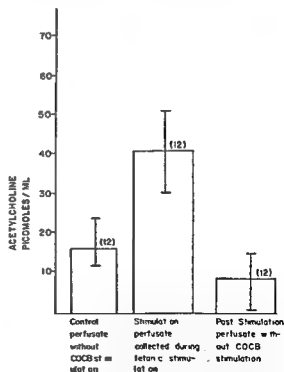


Fig 7 Acetylcholine-like activity released into perilymph. Results are significantly different $p < 0.001$. Tetanic stimulation - 40 shocks 1 msec wide and 200 μ A in amplitude given at the rate of 400 shocks/second.

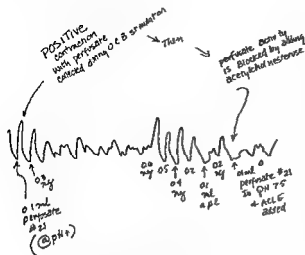


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fluid spaces of the cochlea? 2) If present, is this neurohumor acetylcholine? 3) If present, is the quantity of released neurohumor correlated with COCB stimulation? The experiments depicted in Fig. 6 were carried out because Martini (1940) reported that acoustical stimulation produced ACh-like activity in perilymph collected from pigeons. Our results, on the other hand, indicate that click stimulation does not cause a detectable rise in perilymph ACh-like activity. This incompatibility between our results and Martini's may be based on methodological or species differences.

The significant increase in the acetylcholine-like activity released into the perilymph during COCB stimulation lends strong credence to the cholinergic hypothesis but the absolute amount of neurohumor collected is somewhat disappointing. Eccles (1964) gives an estimate of the acetylcholine output from a single cholinergic fiber as being between 5×10^{-27} and 3.5×10^{-26} grams/impulse. Assuming 1×10^{-26} grams/impulse, 40 impulses per tetanic stimulation, 40 000 fiber endings, and a 90 min perfusion period, then the expected accumulated output of ACh would be 956 pmoles of acetylcholine per perfusion. Actually an average stimulation perfusion yielded only 5 pmoles of acetylcholine-like substance. One of the many possible explanations for this low yield is that the neurohumor is prevented from reaching the perilymph by the basilar membrane. In light microscope preparations the basilar membrane appears as an effective seal between the organ of Corti and the scala tympani and this has been used as an argument in favor of the existence of cortilymph. Such a membrane barrier could easily explain why Bobbin & Konishi (1971) had to use large concentrations of drugs and why the pharmacological evidence did not begin to yield strong positive results until steady perfusion techniques had been utilized. This membrane barrier could also be the cause of the high recovery seen with perfusions of known concentrations of acetylcholine. The new results presented herein, plus the evidence of many of the previous pharmacological investigations

wherein differing results were obtained depending upon whether or not the drugs were given systematically or intracochlearly, therefore imply the existence of blood/cochlea and hair cell/perilymph barriers. These barriers must be taken into account when considering the distribution of the therapeutic and experimental drugs to the inner ear. The experimental results make it most likely that some of released neurohumor does move from the COCB/hair cell junction through the cortilymph and basilar membrane into the perilymph, but some released neurohumor may also diffuse back along the nerve fibers to the habenula perforata and then into the scala tympani. It is unlikely that the released neurohumor diffuses into the scala vestibuli since it must pass the reticular lamina, the tectorial membrane, the endolymph environment of the scala media, and Reissner's membrane before reaching the scala vestibuli. In addition, the large concentration of acetylcholinesterase along the presynaptic nerve terminals will hydrolyse much of the released neurohumor before significant diffusion is possible. The concentration of eserine which may have passed from the perfusion fluid through the basilar membrane may not have been sufficient to block this acetylcholinesterase.

The identification of ACh as the COCB/hair cell transmitter has possible significance with regard to the hypothesized transmitter existing between the hair cells and the afferent nerve terminals. It is unlikely that both the afferent and efferent transmitter substances are the same, since there is a distinct possibility of an interaction of the two in the cleft below the hair cells and ACh has never been shown to enhance afferent nerve activity. Additionally, Jasser & Guth (1972) found an almost complete abolition of choline acetylase activity when the COCB degenerated. Finally, the choline esterase activity is almost completely localized presynaptically on the COCB endings (Iurato, 1971).

In conclusion then, an ACh-like substance is present in the fluid spaces of the cochlea and its concentration is significantly increased during COCB stimulation. Therefore, the criteria for identifying acetylcholine as the chemical media-

tor at the crossed olivo-cochlear nerve/hair cell junction are now largely fulfilled.

ACKNOWLEDGMENT

The authors gratefully acknowledge the assistance of Ms Marion Stockwell who carried out most of the bioassays

ZUSAMMENFASSUNG

Eine Methode zum Nachweis von acetylcholine-ähnlicher Aktivität in der Perilymphe von Meerschweinchen ist entwickelt worden. Diese Aktivität wird durch mässige akustische Erregung unbedeutend erhöht. Sie nimmt aber durch tetanische Erregung vom gekreuzten Olivo-Cochlea-Bündel bedeutend zu. Die Kriterien, um Acetylcholine als den chemischen Vermittler der Verbindung der gekreuzten Olivo-Cochlea Nerven-Haarzellen Verbindung auszusehen, sind nun grossenteils gegeben.

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GENETIC PROGRESSIVE HEARING LOSS IN THE C57/b16 MOUSE

Relation of Behavioral Responses to Cochlear Anatomy

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Abstract Mice (strain C57/b16) were studied using light microscopy. The inner ear developed normally. However, at the age of 3 months, degeneration began in the organ of Corti, beginning in the basal portion and progressing gradually to involve all of the cochlea. By the end of one year, the organ of Corti had disappeared from the basal turn and persisted only in a degenerative form in the apical portion.

Behaviorally, the hearing of this animal was near normal at the age of 2-3 months, except for the frequencies 30 Kc and above. As the animal grew older, hearing gradually deteriorated and the high frequencies were totally lost. The low frequencies were preserved, but at an elevated threshold.

Interestingly enough, at the time when this animal's threshold of hearing above 30 Kc was higher than normal, the organ of Corti appeared normal. Similarly, at the end of one year, light microscopy revealed an atrophic and disorganized organ of Corti present only in the apical turn of the cochlea (the rest of the cochlea having degenerated totally) and yet this animal maintained hearing for the low frequencies although at an elevated threshold. It appears that light microscopy alone cannot discriminate between the functional and non functional state of the cochlea.

The mouse is a particularly good animal for the study of auditory and vestibular systems. There are several species which carry different genes for hereditary deafness and also genes for vestibular degeneration. The auditory system has been most widely studied because of our interest in human hereditary deafness and also because

of the modern advances in the fields of auditory neurophysiology and human communication. The vestibular system in deaf mice has received only limited investigation.

In the study of various sensori neural hearing losses, the aim of investigators has been to relate the behavioral responses of the human to the anatomical findings of the inner ear. The techniques used are based on the early work of Guild (Guild, 1919, 1932). Such material is now more readily available because of the establishment of human temporal bone bank centers. Because of the inherent difficulties of studying the developmental and degenerative hearing losses, our investigators must turn again to animals and try to relate the behavioral and anatomical findings. Such studies are indeed fewer in mice because of the difficulties in the training of these animals to obtain the behavioral puretone thresholds for hearing. Hearing in mice has often been tested by a Preyer reflex—a twitching of the pinna to a sudden tap on a metal pan. Threshold hearing measurements have been made using such an approach (Powers et al., 1966). However, there are several obvious objections to using such methods. In a study published by Birch et al. (1968), a method was developed whereby absolute measurements for pure tone thresholds in the normal CBA-J mice were made using operant conditioning methods with positive reinforcement. The behavioral

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thresholds were compared with physiological data (cochlear potentials, single units from the cochlear nucleus and GSR responses as reported in other studies) (Mikaelian, 1966, Mikaelian et al, 1965, Berlin et al, 1968) and were found to correlate fairly well in the range of frequencies from 10 to 30 Kc. The thresholds were lowest in these ranges. However, there was little correlation of results with frequencies above 30 and below 10 Kc.

The C57/b16 strain of mice was selected for the present study because of the initial observations by Kocher (1960) that these mice hear and respond well in early life (first 2 to 3 months of life) and then gradually lose their hearing as measured by the Preyer reflex. The clinical picture closely resembled certain types of hereditary hearing losses observed in humans. The above observations prompted us to investigate this strain of mice in a strictly controlled experiment and study the developmental behavioral responses and relate the findings to the changes that occurred in the inner ear.

METHOD

The trained subjects were 17 C57/b16 mice bred from stock obtained from the Jackson Laboratory, Bar Harbor, Maine. They were studied in 3 replications (group I $N=5$, groups II and III $N=6$ each) extending over a 28 to 30 month period of time. The apparatus and training procedures are described by Birch and co workers in full detail (1968). A brief description of the method of training is presented here. Training was conducted in a Foringer cage with transparent plastic walls and a grate floor and ceiling. An opaque plastic barrier under which the mouse could walk divided the cage in half. Projecting into the cage from the right wall were a light metal lever and a blunt hypodermic needle, number 20. When the mouse pressed the lever a solenoid was activated so that a drop of sweetened water was automatically dispensed from the tip of the needle.

All decibel readings for the pure tone are relative to 0.0002 dynes per centimeter square

Table I dB SPL output at maximum intensity

Frequency	SPL at 0 Attenuation
2 950	74.8 dB
5 100	83.8
9 800	84.8
19 600	93.8
30 000	100.8
39 500	99.3
57 000	90.8
81 000	79.8
99 500	68.3

(SPL). The experimenter initiated the train of tone pulses which was automatically timed to last 10 sec by means of a clock timer. During this tonal period, the mouse could cross to the right of the cage, press the lever, and lick from the blunt needle. Training began at 4 weeks of age. Each animal was deprived of water for 18 to 20 hours before each session. A 39 500 cycle tone at 55 dB was used for training. The animal was conditioned to press the lever during tone and to withhold pressing during silence.

When the animals were pressing the lever on at least 80% of all tonal intervals threshold testing was begun. Two or three tones were tested during each daily session. There were nine tones in all (2 950, 5 100, 9 800, 19 600, 30 000, 39 500, 57 000, 81 000, and 99 500 cycles per sec). The method of limits descending series was used. The most intense tone was 55 to 60 dB SPL. If no response was made at that intensity the attenuation was decreased by 10 dB until a response occurred or until the maximum intensity was reached. Table I shows the maximum SPL possible for each tonal frequency as measured by a Bruel & Kjaer $\frac{1}{2}$ inch probe microphone near the position of the mouse at the start of each trial. The values of the decibel readings are based on two calibration measurements: the microphone being placed at two different positions near where the mouse would be at the start of the training. Tones were presented in random order. The threshold was defined as 5 dB lower than the least intense sound to which the mouse responded. Threshold testing was continued to 12 months for 6 animals, 16 months for 5 mice and up to 21 months for 6 mice.

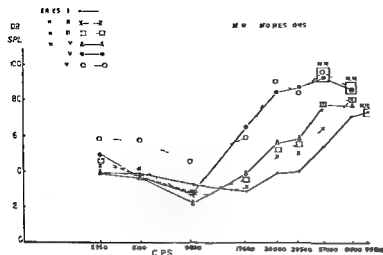


Fig 1 Pure tone threshold changes with age C57/b16 mouse

The threshold SPL for each tone is based on the mean five different measurements. It required 2 to 4 weeks in general to test a complete series of tones five times each. The criterion for eliminating a tone from the testing series was that no response (NR) occurred on four out of the five consecutive measures.

Histological studies

A series of C57/b16 mice were studied histologically (light microscopy) at various ages. The animals were anesthetized with intra peritoneal injection of 0.2 cc of a 20% solution of urethane and perfused with normal saline through the left ventricle followed by 10% formalin (Lilly's).

neutral) The heads were removed trimmed and decalcified and then embedded in celloidin.

They were then sectioned serially at 10 μ m and every fifth section was stained with hematoxylin and eosin and mounted for study.

RESULTS

A Behavioral

Fig 1 shows the changes in pure tone sensitivity with age. Table II shows the threshold values and standard deviations. Thresholds for series I-IV are computed by assigning to each mouse the mean point to the nearest 1-month between his age at the beginning of the five measures and

Table II

T = Threshold
SD Standard dev at on

[illegible]

Table III Mean and age range of the mice

Series	Mean age (months)	Range (months)
Series I	2.28	1.75-3.00
Series II	3.21	2.25-4.00
Series III	3.95	2.75-5.00
Series IV	4.86	3.75-7.25
Series V	10.74	9.00-13.00
Series VI	17.22	14.50-21.50

his age at the end. These mean points were then averaged for all 17 mice (Table III). The mean and age range for each series are given in this table.

The results show a decided progressive high tone loss with age. Sensitivity to tones 9 800 Hz and below show no loss up to the mean age of 10.74 months. By 17.22 months, the losses are between 10 and 20 dB for these tones. The loss is gradual. For frequencies 19 600 cycles and above all show decreased sensitivity between the first and second series, between the second and third series, and between the third and fourth series (except for 57 000 cycles which was the same in the third and fourth series). By 10 months there was no response at 57 000 cycles and above.

Birch and co-workers (1968) published pure tone thresholds for normal CBA-J mice using the same apparatus and training procedures.

Fig. 2 contains a comparison between the CBA-J mice and the series I and Series VI thresholds of the mice of the present study. The age of the

normal CBA-J mice at the initial measurement was 4 to 7 months. The Series I threshold curve diverged at 30 000 cycles and above, indicating that the very high frequency sensitivity of the C57/b16 mice is inferior to that of the normal CBA-J mice at an early age.

B. Histological studies

Two mice of the C57/b16 strain at various ages were sacrificed for histological study. The ages at sacrifice were 1 week, 2 weeks, 3 weeks, 4 weeks, 2 months, 3 months, 5 months, 12-14 months, 18 months, and 19-20 months.

A total of 18 C57/b16 mice were sacrificed. A controlled series of normal CBA-J mice was sacrificed at comparable ages. No degenerative changes were seen in the inner ears up until the age of 3 months. Until then the organ of Corti had a normal structure.

The earliest change seen in the C57/b16 mice was observed at the age of 3 months (Fig. 3). This was apparent first in the basal turn and varied in extent. In some, a disorganized organ of Corti was seen where some of the hair cells were missing, the supporting cells and the pillar cells were clumped together. All structure had a atrophic appearance. Tunnel fibers could still be seen. The stria vascularis was normal, and the ganglion cells were well preserved and had a normal population. In the other turns, the organ of Corti appeared structurally normal.

In the fourth and fifth months (Fig. 4), the

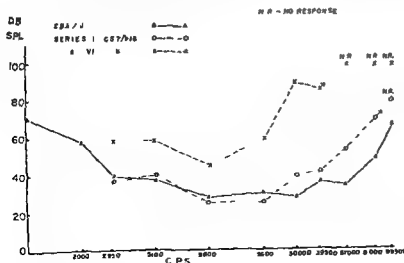


Fig. 2 Comparison of pure tone thresholds between the CBA/J and the Series I and Series VI of the C57/b16 mice.

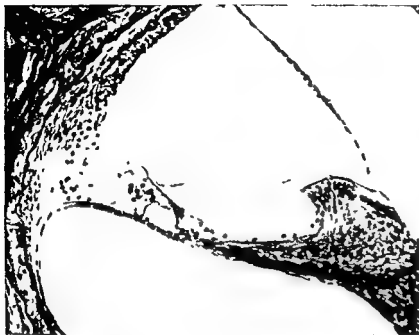


Fig 3 Earliest changes seen in the organ of Corti C57/b16 mouse, age 3 months Animal no 3329, $\times 200$ See text for description

degeneration involved both turns of the cochlea with complete absence of the organ of Corti in the basal part. However, in the uppermost areas the organ of Corti was somewhat disorganized—fewer hair cells were found and atrophic changes could be seen in both the hair cells and supporting cells. The stria vascularis revealed some

atrophy, which was present mostly in the basal area. At this stage, also, the ganglion cells in the basal portion were fewer and showed atrophy.

About the age of one year, the picture was almost uniform (Figs 5 and 6). Absence of the organ of Corti with atrophic stria vascularis, very few neurons in the modiolus in the region



Fig 4 C57/b16 mouse, age 5 months Animal no 3305, $\times 75$ See text for descriptions



Fig 5 C57/b16 mouse 1 year old. Animal no. 3037. $\times 75$. See text for descriptions.

of the basal turn. The apical turn showed a disorganized organ of Corti with atrophic hair cells and supporting cells (Fig 7). The neuron population was still preserved but slightly reduced.

DISCUSSION

The purpose of the animal investigation was to relate the degenerative changes of the Corti occurring in the C57/b16 maturing mouse with



Fig 6 C57/b16 mouse. Same as Fig 5. High power view. $\times 200$. Basal turn.

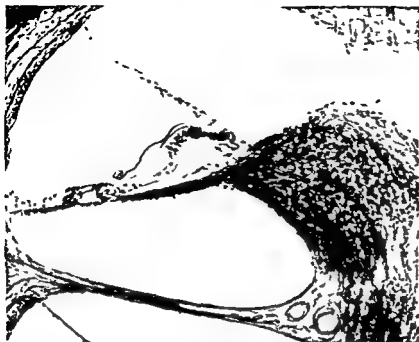


Fig 7 C57/b16 mouse Same as Fig. 6, $\times 150$ Apical turn See text for description

hearing loss determined by pure tone thresholds. Deterioration of hearing began with the high frequencies and gradually involved the lower ones, but the animals never became totally deaf. Considered anatomically, degeneration in the organ of Corti became apparent first in the basal portion of the cochlea at the age of 3 months, and gradually extended to the apex. The pattern of degeneration of the organ of Corti consisted of disorganization of the organ of Corti and the hair cells, with atrophy setting in, and gradually the organ of Corti completely disappearing. The spiral ganglion cells diminished progressively. In the apical area of the cochlea the organ of Corti persisted in a dispersed manner, but one could still identify various atrophic hair cells and supporting cells. The spiral ganglion cells were maintained in the upper part. The stria vascularis became smaller and atrophic in the areas where the organ of Corti had completely degenerated. The cochlear duct and scala media were preserved normally throughout. The vestibular part of the labyrinth persisted normally.

For the behavioral results the C57/b16 mice did not appear to possess normal thresholds for

hearing in the frequencies of 30 000 cycles and above, as compared with the CBA-J mice (presumed to be normally hearing mice). The discrepancy was observed even before degenerative changes could be observed in the cochlea. The picture resembled the previously reported observations in the Shaker-1 mouse, where this animal had lost hearing totally by the 22nd day after birth, and yet the organ of Corti appeared absolutely normal (Mikaelian & Ruben, 1964). On the other hand the low frequencies still persisted in the C57/b16 mice, though with an elevated threshold. The organ of Corti in the apical portion of the cochlea appeared degenerated, yet this animal maintained hearing. It must be that the remaining hair cells of the organ of Corti were still functioning in this area. Of interest would be electronmicroscopic studies of the hair cells in this region and to try to determine the nature of innervation of these cells. It is of great interest that such a disorganized organ of Corti was still functioning, as shown by the behavioral pure tone thresholds.

If one were to make a general statement as to the pattern of degeneration, the direction of the affection was from base to apex, and it



Fig 5 C57/b16 mouse 1 year old. Animal no. 3037. $\times 75$. See text for descriptions.

of the basal turn. The apical turn showed a disorganized organ of Corti with atrophic hair cells and supporting cells (Fig 7). The neuron population was still preserved but slightly reduced.

DISCUSSION

The purpose of the animal investigation was to relate the degenerative changes of the Corti occurring in the C57/b16 maturing mouse with



Fig 6 C57/b16 mouse. Same as Fig 5. High power view. $\times 200$. Basal turn.

THE EFFECT OF PRESERVATION ON THE BEHAVIOUR OF HOMOLOGOUS OSSICULAR GRAFTS

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(Received August 6, 1973)

Abstract The present study deals with the effect of several preservation methods on the behaviour of incus grafts in rats after orthotopic and heterotopic transplantations. The following conclusions can be drawn: 1. Preservation seems to influence both the potential for new bone formation and the antigenic properties of an ossicular graft. 2. Preservation in cialit and alcohol and freeze drying preserves the osteogenic properties to a variable extent. 3. Bone formation is absent after boiling and after preservation in formaldehyde. 4. The antigenic properties are affected by storage in alcohol and cialit and by boiling. 5. Preservation of the antigenic properties of the graft does occur after freezing and freeze drying and after storage in formaldehyde. 6. Typical reaction of transplantation and resorption of the graft are only observed after heterotopic transplantation in muscle.

From clinical experience we know that ossicular transplants for tympanoplasty are well tolerated in the middle ear. There are no definite reports indicating that these transplants are extruded or rejected like true organ transplants in other parts of the body. However, the lack of understanding of the fundamental reasons underlying this, demands a critical attitude towards their use. Many basic biological problems remain to be solved. Of these most important ones are the problems concerning the possible antigenicity of these grafts and the problems of revitalisation and resorption.

From experiments in animals it has become clear that reactions against either fresh or preserved homologous grafts do not seem to occur in the middle-ear or at least are so weakened that they cannot be traced by the methods used up to date. When a fresh homologous incus is trans-

planted into muscle signs of a transplantation reaction become visible which shows the antigenicity of such a graft (Van den Broek, 1968, Kuijpers & Van den Broek, 1972) and the question arises whether in the long run any inferior behaviour in the middle ear could result from the preservation of these antigens. Experiments by Kastenbauer (1972) have learned that the antigens from an ossicular graft can elicit a second set reaction in the middle-ear of previously sensitised animals.

A closely related problem is the influence of preservation on the revitalisation of the graft. From the literature we know that there is no consensus of opinion on the degree of bone formation in ossicular grafts (Wilson et al., 1966, Smith & Overton, 1968, Benitez & McIntire, 1968, Dawkins, 1971, Kerr & Smyth, 1971).

The aim of this study has been to study the effect of different preservation methods on both the antigenic properties of an ossicular graft and on the potential for the formation of new bone. Because boiling of an ossicle denatures all the proteins in a graft and, as shown in previous experiments, abolishes the potential for the formation of new bone, this method is not included.

Both orthotopic and heterotopic transplantations were performed. Heterotopic transplantation in leg muscle has the advantage of a more intimate contact between graft and host tissue which will result in more pronounced effects than in the middle-ear.

Table I *Summary of the experiments performed*

Preservation method	Preservation time	No of rats
Alcohol 70 %	1 week	14
	9 weeks	9
	19 weeks	4
Formalin 4 %	1 week	10
	8 weeks	8
	17 weeks	8
Cialit	1 week	8
	2 weeks	5
	7 weeks	8
	20 weeks	7
Freezing	33 weeks	4
Freeze drying	2 weeks	10

MATERIALS AND METHODS

Homologous transplantations of incudes were performed between the middle ears of two genetically different strains of young adult rats. The incudes were removed from one strain, preserved in different ways for varying periods of time, after which they were transplanted into the middle ear of the second strain. At the same time heterotopic implantation of incudes preserved in the same way was performed in muscle. All grafts were rinsed in saline before introduc-

tion into the middle ear and muscle, except those preserved by freeze drying.

Table I shows a schematic representation of the different types of preservation used as well as the preservation time and the number of experiments performed. After varying survival time the animals were sacrificed, the middle ears and muscle fragments containing the grafts removed and processed for microscopical study as described previously (Van den Broek & Kuipers, 1967). From the serial sections the most important features (overall vitality, formation of bone reaction of the recipient site, and the degree of resorption) were recorded and tabulated.

Alcohol

Alcohol 70 % has been used by many authors as a preservative for ossicles. Its biological effect is dehydration, which results in coagulation of the proteins, while the acidic or basic character of the proteins remains unaltered. Its antiseptic qualities have been known for a long time and add to its usefulness as a preservative for grafts.

In Table II the results of this group of experiments are recorded. The local reactions towards an alcohol preserved implant are minimal. The

Table II *Preservation in alcohol*

Res = resorption; Ot = otitis media

Survival time	1 week				9 weeks				19 weeks			
	Ear		Leg		Ear		Leg		Ear		Leg	
	New bone	Res	New bone	Res	New bone	Res	New bone	Res	New bone	Res	New bone	Res
10 days	-		-		-	-		+			-	-
10 days					-	-		+				
3 weeks			-	+	-	-		++		-		-
4 weeks				+	-	-		++				
4 weeks			+	+								
4 weeks				++								
4 weeks				++								
3 months		-		+								
3 months		-		++								
3 months		-		++								
3 months		-	+	+								+
4 months	+		+	+	-	-		++				
4 months				+	-	-	+	++				
12 months					+			++				
12 months					+	Ot		++				
12 months					+	-	+	++	-		+	++



Fig 1 Cialit preserved (7 weeks) incus in muscle after 18 months. Note small areas of new bone formation (→)

vitality of the implant is entirely lost after the storage in alcohol and during the first weeks the incus appears entirely dead. Formation of new bone was first found after 4 weeks. From 3 months onwards replacement by new bone is more marked especially in the heterotopic grafts, which in nearly all cases showed signs of osteogenesis. In the middle-ear only 7 grafts were found with signs of bone formation. This bone formation, however, has been rather scanty. There was no relation between the time of storage and the degree of osteogenesis appearing in these grafts. Even after storage for 19 weeks, bone formation was observed. Although resorption was noted in muscle, this was never found in the middle ear.

Cialit

Cialit is an organic mercury compound with bactericidal and fungicidal properties. It has been used since 1954 in orthopaedics for preservation of bone and was first used in otology by Marquet (1966) as a preservative of homograft drum membranes. For these purposes a solution of 1:5000 in distilled water is used.

In Table III a survey of the experiments is recorded together with the most important findings regarding bone formation and resorption. It can be seen that also after this type of preserva-

tion new bone can be found in the incus, which is also slightly more pronounced after heterotopic transplantation. The reaction of the recipient site at 10 days consists of a minimal cellular infiltration which has disappeared after a fortnight and compares very well with the reaction around the alcohol preserved grafts.

The formation of new bone in these grafts was generally restricted to small areas (Fig 1). In one of the specimens the narrow spaces looked wider than normal, otherwise no signs of resorption were found in the middle-ear. In muscle, moderate resorptive activity was found. No significant differences between alcohol- and cialit-preserved grafts became apparent and the behaviour of ossicular grafts after preservation and in cialit or alcohol seems to be very similar.

Formaldehyde

Incus grafts were preserved for 1, 8 and 17 weeks in formaldehyde, 4% solution, buffered at pH 7. In contrast to alcohol, formaldehyde is a non-coagulant fixative and many of the hydrophilic groups retain their relation with water molecules.

The histologic findings are summarized in Table IV. It can be seen that in none of these grafts was formation of any new bone found. One of the most remarkable findings of these experiments has been the persistence of the donor



Fig. 2 Formaldehyde-preserved (8 weeks) incus in the middle ear after 4 months. Most osteocyte nuclei are still present.

osteocyte nuclei in these formalin fixed grafts even after prolonged time in the recipient (Fig. 2). With all the other preservation methods, total disappearance of the donor osteocyte nuclei has occurred within a fortnight.

In the middle-ear no reaction to the grafted ossicle was present. In muscle, quite extensive resorption was apparent after 4 months, illustrated by the presence of a considerable number of osteoclasts. Furthermore, collections of mononuclear cells have been seen around these grafts, starting with the shortest survivals and persist-

ing, although to a lesser extent, throughout the observation period (Fig. 3). These findings are highly suggestive of transplantation phenomena as observed when fresh homologous grafts are used (Kuypers & Van den Broek, 1972). The absence of this reaction in the middle-ear is also in agreement with earlier findings when fresh homografts failed to reveal any reaction in transplantation in the middle-ear as opposed to fresh grafts in muscle.

In order to exclude any possible role of formaldehyde as an aspecific stimulus causing a mono-



Fig. 3 Formaldehyde-preserved (8 weeks) incus in muscle after 4 months. Extensive monocellular reaction is clearly visible.

Table III *Preservation in cialit*

Res = resorption Ot = otitis media

Survival time	1 week				2 weeks				7 weeks				20 weeks			
	Ear		Leg		Ear		Leg		Ear		Leg		Ear		Leg	
	New bone	Res	New bone	Res	New bone	Res	New bone	Res	New bone	Res	New bone	Res	New bone	Res	New bone	Res
10 days		-		±	-		-									
10 days				±		-		±						-		
2 weeks			-	+	-		-				-					
4 months	-			+		-		-								
4 months				+									±	-	Ot	
4 months	±		+	+												
4 months	±	-	+	+	+		+								-	-
4 months	±			+												
7 months	-			-					±		-	-				
12 months		-		+												
12 months	+		+	+											-	-
18 months									+	-						±
18 months											+					

cellular reaction, additional experiments were performed by first boiling the incudes and subsequently storing these in formaldehyde for 1 week. No monocellular reaction was found around these incudes after about 3 months.

Storage at low temperature

Another method of preservation is storage at temperatures below 0°C. Two different methods have been used in these experiments. By the first the incudes were put into distilled water

immediately after removal, frozen to -25°C and then stored at the same temperature (freezing). By the second method these incudes were frozen immediately to -85°C in solid carbon dioxide and consequently lyophilised at -30°C and stored for 2 weeks at the same temperature (freeze-drying). Because the storage time in both groups was very different (33 v. 2 weeks) the results are only partly comparable.

These two groups of experiments are summarised in Table V. No bone formation was found

Table IV *Preservation in formaldehyde*

Res = resorption React = reaction consisting of mononuclear cells

Survival time	1 week						8 weeks						17 weeks					
	Ear			Leg			Ear			Leg			Ear			Leg		
	New bone	Res	React	New bone	Res	React	New bone	Res	React	New bone	Res	React	New bone	Res	React	New bone	Res	React
7 weeks				-	+	+							+	-				
2 weeks						+												
2 weeks						+												
4 weeks										-								
4 months										-								
4 months													+	+	+			
4 months													+	+	+			
13 months					+													
13 months					+	+				-				+	+			
13 months					+	+								+				

Table V Preservation by freeze drying and by freezing

Res = resorption React = reaction consisting of mononuclear cells Ot = otitis media

Survival time	Freeze drying						Freezing					
	Ear			Leg			Ear			Leg		
	New bone	Res	React	New bone	Res	React	New bone	Res	React	New bone	Res	React
2 weeks	-	-	-	-	-	-	-	-	Ot	-	++	+
2 weeks	-	-	Ot	±	±	±	-	-	-	-	-	-
4 weeks	++	-	-	-	++	++	-	-	-	-	-	-
4 weeks	++	-	-	-	++	++	-	-	-	-	-	-
4 months	++	-	-	+	++	+	-	-	-	+	+	+
4 months	+	-	-	-	+	-	-	-	-	-	+	±
9 months	+	-	-	-	+	±	-	-	-	-	+	+
14 months	+	-	Ot	-	+	±	-	-	-	-	++	+
14 months	-	-	-	-	++	+	-	-	-	-	-	-
14 months	-	-	-	+	++	±	-	-	-	-	-	-

in incudes in the middle ear when these were frozen. In muscle in only one of the incudes was bone formation observed. Incudes which were freeze dried and kept for 2 weeks were found to show bone formation in most cases after 4 weeks (Fig. 4). In muscle the result was less prominent and bone formation was found in only three of the specimens. As to the reaction around these grafts one can see that in both groups marked monocellular reactions were present to an extent not observed around any of the ear grafts except in those which were stored in formaldehyde (Fig. 5). It is reasonable to assume

that this monocellular reaction also depends on the preservation of antigenic properties when this method of storage is used.

Decalcification

Decalcification of both fresh incudes and incudes stored for 2 days in alcohol or formaldehyde was performed in EDTA (10%, pH 7.0) for 10 days at 4°C. Subsequently the incus was transplanted. At histological examination the incudes were found to be infiltrated by connective tissue cells (Fig. 6). No difference could be observed between the behaviour of the fresh and the pre-

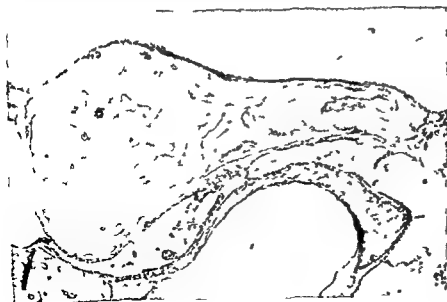


Fig. 4 Freeze-dried incus in the middle ear after 9 months. Area of new bone in the long process of the incus.



Fig 5 Freeze-dried incus in muscle after 4 weeks. Note the monocellular reaction present around the tip of the incus.

served grafts. The infiltration by connective tissue cells was much more pronounced in muscle than in the middle ear. It was quite remarkable that hardly any osteoclasts were observed in the incuses in muscle even after longer survival times which contrasts markedly with all the other experiments performed. Often fat was found surrounding the incus in the muscle and sometimes replacing part of the graft. In the middle-ear no resorptive activity was found up to one year.

DISCUSSION

The findings of the present study indicate that the behaviour of a grafted ossicle both in the middle ear and in muscle is related to the mode of preservation.

Two properties of the graft seem to be influenced by the preservation technique. First the potential for remodelling by new bone and second the antigenic action of the graft. These processes are at least partly independent.



Fig 6 Incus decalcified with EDTA transplanted into the middle ear. Results after 12 months. Incudo-malleal joint.

From the results obtained it appears that storage in alcohol, cialit and freeze drying (and presumably also freezing) preserves the potential for new bone formation. However, this new bone formation mainly confined to the central parts of the grafts, starts much later than in fresh auto- and homografts. In the latter cases this new bone visible within a few days after transplantation, is probably formed by surviving periosteal cells of the graft (Van den Broek & Kuypers, 1967).

When the ossicles are stored in formaldehyde or denatured by boiling or drying as previously reported (Van den Broek, 1968) no bone formation has been found, even after prolonged presence in the middle-ear or in muscle.

From the available data it follows that new bone formation in preserved ossicles is dependent on host cells, which are probably transformed into osteogenic cells under influence of the graft. No conclusion can be drawn from the character of the substances in the graft which are responsible for this transformation process. The preservation methods after which new bone formation was found have in common that the original molecular structure of the tissue components is more or less retained, although after preservation in formaldehyde which also preserves the molecular structure very well, bone formation was absent.

It is noteworthy that careful decalcification at neutral pH with EDTA appeared to destroy this potential in alcohol-preserved specimens. In addition the decalcification seems to change the structure of the organic matrix in such a way that connective cells can easily penetrate into the graft between the fibres.

The second problem which appears to be related to the preservation method is the antigenicity of the graft. It seems that the antigenic properties of the graft are preserved after storage in formaldehyde, freeze drying and freezing, but not after storage in alcohol or cialit, or after boiling, as far as could be detected by the methods used. This was concluded from the presence of a characteristic monocellular reaction around the grafts implanted in muscle sim-

ilar to that observed previously around fresh homologous grafts in this location (Kuypers & Van den Broek, 1972). These findings do suggest that those preservation methods which do not alter profoundly the nature of the proteins, do preserve at least some of the antigenic properties. The absence of a monocellular reaction towards these grafts in the middle-ear confirms the previously stated hypothesis that the middle ear reacts differently and that these processes, if present, are very much mitigated or retarded. Related to this problem, experiments have been performed by Kastenbauer (1972), who observed reactions suggestive of a transplantation reaction in the middle-ear only after previous sensitization (second set phenomenon), which might mean that transplantation immunity in the middle-ear is not at all to be ignored. Hitherto, however there is no clear evidence that the clinical results of using preservation methods which, according to our experiments, seem to preserve antigenic properties are any inferior (Perkins, 1971), although the inferior results reported by Smyth (1972) with freeze-dried homograft drums might be significant.

In conclusion, there is no doubt that the behaviour of a dead preserved ossicle is a dynamic one, the ultimate fate of which is difficult to predict. Therefore, in using these ossicles, one should take into account the above-mentioned effects of the various preservation methods.

ZUSAMMENFASSUNG

Das Verhalten konservierter homologer Amboss-Transplantate nach orthotopischer und heterotopischer Transplantation wird im Tierexperiment untersucht. Zusammenfassend könnte folgendes festgestellt werden: 1. Konservierung beeinflusst die osteogene Potenz und die Antigenität der Transplantate. 2. Aufbewahrung in Cialit und Alkohol und Einfrieren und Lyophilisieren konserviert in verschiedenem Masse die osteogene Potenz. 3. Diese Potenz wird völlig destruiert durch Kochen und Aufbewahren in Formalin. 4. Die Antigenität wird durch Kochen und Aufbewahrung in Alkohol und Cialit beseitigt. 5. Einfrieren, Lyophilisieren und Konservieren mit Formalin bewahrt mindestens ein Teil der Antigenität der Amboss-Transplantate. 6. Abwehrreaktionen gegen homologe Amboss-Transplantate werden nur beobachtet nach heterotopischer Transplantation in Muskeln.

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OTITIS EXTERNA AND ALLERGIC CONTACT DERMATITIS

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Abstract Among 98 patients with otitis externa 34 (35%) gave positive response to patch tests. The most common finding was allergy to drugs, as a rule neomycin. On the basis of the study it is concluded that the incidence of allergic contact dermatitis is 12-22% among patients with otitis externa.

Otitis externa designates a number of different disease conditions of the auditory canal of uncertain aetiology and pathogenesis. The disease is of great practical importance. Singer & Freeman, in 1952, found that the *Pseudomonas* group was the most important aetiological agent. According to their investigations fungi played a limited aetiological role. In Jones' (1971) opinion a primary factor is the development of allergy to bacteria and fungi. Baumann & Carr (1961) were able to disprove the theory that cerumen from normal ears possessed antibacterial properties as compared with cerumen from ears affected with otitis externa.

The aetiology is presumably multifactorial. Local factors, such as temperature and humidity, are important. Infection is probably in most cases secondary. The topical use of antibiotics may convert an "idiopathic" eczema into allergic contact dermatitis. Lastly, the aetiology may be primary contact dermatitis. McKelvie & McKelvie (1966) found 5 cases of allergy in a study of 113 patients, but their patients were tested only for chrome, nickel, and neomycin.

The present study was designed to elucidate the incidence of primary and secondary allergic contact dermatitis in patients with otitis externa.

METHOD AND MATERIAL

The material comprises 100 consecutive patients referred to the E N T Department by otological practitioners. To avoid a preponderance of particularly severe cases, the referring otologists were requested to refer all their cases of otitis externa to us during the period concerned. No selection was made.

The history-taking and physical examination were carried out and recorded according to a fixed plan. The diagnosis of otitis externa was based on the patients' symptoms in the form of itching, discharge, and pain and on the objective findings. Thereafter, swabs were taken from the auditory canal. The Department of Dermatology carried out an examination for fungi, standard eczema tests, patch tests with sulphathiazole cream, HgCl₂, shellac, Furacin® (iodoform Otosafanal (procaine chloride), Ciloprin® (4-carboxymethylamino-4-aminodiphenylsulfone) and patch tests using the drugs that the patient has had for treating the otitis externa. Finally, patch tests of the patient's cosmetics were applied. The patients attended again for reading of the patch tests. No therapeutic trials or follow-up were done.

Of the 100 patients 62 were females and 38 males. Two patients failed to appear after the patch tests had been applied and were therefore excluded. Two thirds of the patients had been suffering from their disorder for more than 2 months. About two-thirds had previously had

Table I Distribution of the 57 cutaneous allergies found in 34 patients with otitis externa The allergy was demonstrated by positive patch test

Substance	Number
1 Drugs	30
Neomycin sulphate	8
Coal tar	4
Methylolate* (preservative <i>int al</i> in Synalar*)	3
Wood tar	3
Mercuric chloride	2
Nitrofurazone (Furacin*)	2
4-carboxymethylamino-4-aminodiphenyl sulfone (Clotrim*)	1
Chloramphenicol	1
Chlorquinaldol (Sterosan*)	1
Dequalonium chloride (Danical*)	1
Paraben (preservative)	1
Polymyxin sulphate	1
Procaine chloride (Oto-afanal*)	1
Iodochlorhydroxyquin (Vioform*)	1
2 Chemicals	12
Formaldehyde	2
Shampoo and hair dressings	2
Aminozobenzene	1
Gum	1
Colophonium	1
Adhesive plaster	1
Paraphenylenediamine	1
Balsam of Peru	1
Turpentine	1
Hearing aid inserts	1
3 Metals	10
Cobalt chloride	4
Nickel sulphate	4
Potassium bichromate	2
4 Miscellaneous	5
Chrysanthemum	2
Primrose	2
Orange	1

otitis externa. The majority of the patients had been treated by their own otologist, many of them with little effect.

RESULTS

In 34 out of 98 patients with otitis externa one or more cutaneous allergies were found (35%). Both sexes were equally represented. A total of 57 allergies were found in these 34 patients. Table I shows the distribution of the positive patch tests.

The largest group is made up of drugs. It will

Table II Patients' assessment of the effect of treatment given

$n=34$ in the allergy group and $n=64$ in the non allergy group $p<0.05$

	Allergy	No allergy
No effect	88% (28)	67% (37)
Good effect	15% (5)	23% (18)
Not treated	(1)	(9)

be seen that a risk of sensitization is involved by nearly all agents for topical application into the auditory canal. Like others before us, we also found neomycin allergy to be common. Otherwise, it is not possible to deduce from the tabulation which agents carry the greatest risk of sensitization. The second largest group consists of various chemicals. Paraphenylenediamine is used *inter alia* in hair dyes. The metal allergies are of interest to the otologist in connection with irrigation by metal syringes after which iatrogenic otitis externa has been observed and in the use of hairpins and matches for scratching the ear.

In an effort to demonstrate differences between the allergic group and the patients in whom no allergy was found, the following factors were investigated:

- 1 Effect of the treatment given
- 2 Site of the eczema
- 3 Duration of the disease
- 4 Symptoms
- 5 Tendency to relapse
- 6 Bacteriological findings

Table III Site of lesions in the two groups. The allergic contact dermatitis is more apt to spread on the auricle

$p<0.05$ $n=34$ and $n=64$

	Allergy	No allergy
Auditory canal only	48% (16)	88% (43)
Auditory canal and auricle (in some cases retroauric)	52% (17)	32% (20)
Unknown	(1)	(1)

Table IV *Distribution of symptoms in the two groups* There is no difference between the non-allergic and the allergic group with respect to the subjective symptoms

($n = 34$ and $n = 64$)

	Allergy	No allergy
Itching	100% (34)	92% (59)
Discharge	71% (26)	75% (48)
Pain	42% (16)	39% (25)

Table II presents the patients' own assessment of the treatment given. Among the patients with diagnosed allergy 28 (85%) reported no effect, in the non-allergic group 37 (67%). The difference is fairly significant ($p < 0.05$).

With respect to the site, Table III shows that allergic contact dermatitis is more apt than non-allergic eczema to spread on the auricle ($p < 0.05$).

It is not possible to decide, on the basis of the patients' symptoms, whether the condition is possibly interpretable as a development of allergy. Table IV shows no difference between the two groups.

There was no significant difference between the two groups with respect to duration or tendency to recurrence (Tables V and VI).

Concerning the bacteriological findings (Table VII), one might have expected to find *Pseudomonas* more often in the non-allergic group. On the other hand, an allergic contact dermatitis might get infected, and a large number of the patients had been or were being treated.

Lastly, pityriasis simplex was demonstrated in 10 patients, all of the group without demonstrable allergy.

Table V *Duration of the disease in the two groups*

($n = 34$ and $n = 64$)

	Allergy	No allergy
More than 2 months	76% (26)	66% (42)
Less than 2 months	24% (8)	34% (22)

Table VI *Number of patients with a history of previous otitis externa* There was no greater tendency to relapse in the allergic group

($n = 34$ and $n = 64$)

	Allergy	No allergy
Previous history of otitis externa	62% (21)	56% (36)
No history of previous otitis externa	38% (13)	44% (28)

DISCUSSION

The disease is common and often long lasting. Therefore, correct treatment is important. It has been demonstrated, for instance, that treatment by a combined agent, such as flumethasone pivalate + iodochlorohydroxyquinoline, is no better than placebo (Brahe Pedersen & Osterhammel 1971), and it is of the utmost importance not to use a drug which maintains or aggravates the disease, because of secondary development of allergy. This possibility must be borne in mind in the event of lacking therapeutic effect or a localization outside the auditory meatus. The use of neomycin preparations is particularly fraught with risk, but the possibility is present with all drugs. Even steroids may sensitize due to their content of preserving agents, but Coskey (1965) reported two cases of allergy to hydrocortisone.

The real incidence of allergic contact dermatitis among patients with otitis externa is prob-

Table VII *Bacteriological findings in swab material from the auditory canal in the two groups*

In some patients there was more than one bacteriological finding

Bacterium	Allergy	No allergy
<i>Staph. albus</i>	11 (32%)	22 (34%)
<i>Staph. aureus</i>	16 (47%)	24 (38%)
<i>Str. faec.</i>	4 (12%)	16 (25%)
<i>Pseudomonas aeruginosa</i>	11 (26%)	19 (30%)
<i>E. coli</i>	1 (3%)	8 (13%)
<i>Proteus</i>	—	5 (8%)
<i>Phaefter</i>	—	1 (2%)
Miscellaneous	3 (9%)	7 (11%)

ably not 35%. Comparison of the history with the allergies demonstrated gave the following findings

1 In 12 of the 34 patients there was conformity between the allergy and the history. The patients had observed flare-up or aggravation in connection with exposure.

2 Ten patients had not observed any connection. However, the agent concerned had been used and had failed to improve the condition.

3 In the case of the last 12 patients there were no data in the history or else the allergy demonstrated could hardly be a contributory factor.

According to the present study, allergic contact dermatitis occurs in 12–22% of patients suffering from otitis externa (group 1 + group 2).

ZUSAMMENFASSUNG

Bei der Untersuchung von 98 an Otitis externa leidenden Patienten ergab eine Auswertung positive Lappchenproben bei 34 (35%). Die häufigsten Befunde waren medikamentelle Allergie, unter diesen Neomycin. Auf Grund

der Untersuchung wird geschlossen, dass die Häufigkeit des allergischen Kontaktekzems bei an Otitis externa leidenden Kranken zwischen 12 und 22% liegt.

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playing the sequence of intervals closely along the horizontal axis (for example, see Fig 2a) This display was found to present a sensitive visual representation of the variations of inter-saccadic interval pattern As seen in this figure, peaks in the interval histogram tend to be associated with horizontal layers in the sequential display In cases in which the interval statistics show strong trends, as in caloric nystagmus (Figs 6 and 7) the sequential display often makes the trend readily visible and suggests how best to segment the data for histogram analysis (Fig 8)

RESULT

The pattern of inter saccadic intervals of a spontaneous nystagmus recording is shown in Fig 2 The record was obtained from a patient suspected of having a labyrinthine lesion, nystagmus was found in all nine head positions in a routine clinical test In Fig 2a, two characteristics of the inter-saccadic intervals can be observed (1) a weak trend in the interval durations is seen by the more frequent occurrence of the short intervals at the beginning of the record, whereas more intervals of longer duration occur in the second half of the record, and (2) the dots have a tendency to cluster into two layers, especially in the

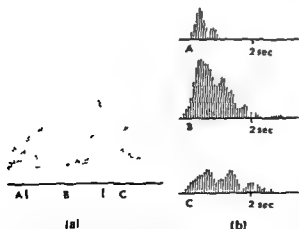


Fig 3 Data from positional nystagmus (a) Sequential display of interval duration 370 samples (b) Interval histograms (A) 45 samples (B) 210 samples, (C) 115 samples



Fig 4 Data from alcoholic nystagmus (a) Sequential display of interval duration 135 samples (b) Interval histogram 135 samples

left half of the record This is reflected in the multi modal interval histogram obtained from the left half of the data and shown in Fig 2b The values of the second and third modes of the histogram are approximately twice and three times the value of the first mode

The results of analysis of positional nystagmus from another patient, suffering from post traumatic vertigo, are shown in Fig 3 Nystagmus was recorded in the caloric test position with the head tilted 30° to the right The trend in this record is quite pronounced especially at the beginning of the record It reflects the fact that the nystagmus was quite rapid when the head was first turned to the right, but gradually settled to a lower frequency The nystagmus recording shows a corresponding decline in intensity A gross layering effect can be seen in the sequential display, and histograms of the segmented data all show multi modal distributions The values of the higher order modes are approximately integral multiples of the value of the basic mode in each histogram even though the basic mode clearly increases with time

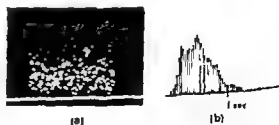


Fig 5 Data from spontaneous nystagmus (a) Sequential display of interval duration 250 samples (b) Interval histogram 250 samples

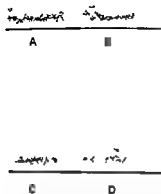


Fig 6 Data from caloric nystagmus of a patient Sequential display of interval duration (A) Left cold (B) right cold (C) left hot (D) right hot Cold = 30°C hot = 44°C water irrigation 30 seconds nystagmus recorded for 90 seconds after irrigation

The results in Fig 4 were obtained from a record of alcoholic positional nystagmus. The subject, who weighed 150 pounds was found to be free from spontaneous positional or gaze nystagmus before the experiment. Six ounces of 100 proof whisky were drunk by the subject within three minutes. Thirty minutes later head turning to the left and to the right resulted in left and right beating nystagmus respectively which were recorded and analyzed. Nystagmus was not so intense as that found in the first two cases and was often interrupted by erratic eye movements.

So far the records have been selected to

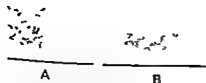


Fig 7 Data from caloric nystagmus of a normal subject Sequential display of interval duration (A) Left cold (B) right cold Nystagmus recorded for 180 seconds after irrigation

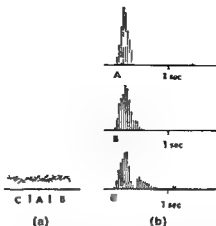


Fig 8 Segmentation of data from caloric nystagmus (a) Sequential display of interval duration taken from Fig 6A (b) Interval histograms of segmented data (A) 60 samples (B) 70 samples (C) 75 samples

demonstrate the existence of multi modality. However, in all three types of nystagmus namely spontaneous positional and alcoholic, there are records in which multi modality is not observed and the interval histograms are mono modal with varying dispersion. For example Fig 5 shows the results from a spontaneous nystagmus record.

In caloric nystagmus due to the rapid change in the intensity of nystagmus multi modality can generally be observed only at the beginning of the response. Fig 6 shows sequential displays obtained from responses to four routine caloric irrigations in a patient suspected of having a central lesion. The layering effect at the beginning of the first three records can clearly be observed. The height of the top layer is approximately twice that of the bottom layer. Fig 7 shows the results from two cold water irrigations in a normal subject. The subject was free from spontaneous positional or gaze nystagmus. Again layering effects at the beginning of the records are observed.

In order to show that the characteristic change between interval histogram and nystagmus intensity (Cheng 1972; Cheng & Outerbridge 1973b) also exist in vestibular nystagmus the data from the left cold irrigation of Fig 6 was segmented and the corresponding

interval histograms are shown in Fig 8 Fig 8 A corresponds to the middle portion of the nystagmus record where the nystagmus intensity was the highest The interval histogram is mono modal and symmetric Fig 8 B corresponds to the end portion of the nystagmus record where the intensity of nystagmus had declined from the peak value The interval histograms is mono modal but skewing towards the longer duration Fig 8 C corresponds to the beginning portion of nystagmus where the intensity was the weakest The interval histogram shows clearly the multi modal phenomenon

DISCUSSION

Analysis of the inter saccadic intervals of caloric nystagmus indicates that the characteristic pattern of change of the interval distribution with intensity found in optokinetic nystagmus (Cheng, 1972, Cheng & Outerbridge, 1973 b) also occurs in vestibular nystagmus As nystagmus intensity decreases, the interval histogram changes from symmetric mono-modal, to asymmetric mono modal and finally to multi modal

In spontaneous, positional and alcoholic nystagmus, the inter saccadic interval histogram is mainly mono-modal with skewness towards the longer interval side or multi modal, whereas symmetric mono modal histograms have rarely been observed This may be due to the fact that in the records so far examined, nystagmus intensity has rarely reached that observed in caloric nystagmus It was also found that in some records (including caloric nystagmus), no multi-modal phenomenon was apparent in the entire record Again nystagmus intensity may be the reason It has also been shown that certain types of mental activity can have a randomizing effect on the saccadic rhythm (Cheng 1972)

The systematic variation of interval histogram with nystagmus intensity, clearly demonstrated in the case of optokinetic nystagmus (Cheng 1972, Cheng & Outerbridge, 1973 b)

is also present in vestibular nystagmus together with the occurrence in both cases of multi modal histograms in which the modes are equally spaced, are of great interest Not only do these findings further emphasize that nystagmus generation mechanisms in the vestibulo ocular and optokinetic systems are very closely related, but a specific proposal can be made as to the type of neural mechanism which may be involved

Multi modal interval histograms similar to those described here have been observed in several neural systems (Poggio & Viernstein 1964, Herz et al, 1964, Bishop et al, 1964). Bishop et al suggested that multi modality could occur if a quasi periodic excitatory action potential process interacted synaptically with a random inhibitory process which intermittently blocked transmission of an excitatory action potential It will be noted that in such an interaction the output interval histogram would become mono modal if the mean frequency of the inhibitory process were reduced It is quite plausible to postulate a similar interaction in the nystagmus generation mechanism, although the single inhibitory and excitatory neurons in the model of Bishop et al would have to be replaced by groups of neurons, and the output action potential by a burst corresponding to each saccade Such a model has been found to be capable of simulating observed variations in the interval histogram of vestibular and optokinetic nystagmus (Cheng, 1972, Cheng & Outerbridge, 1973 a)

ZUSAMMENFASSUNG

Mittels Digitalrechner wurden die Zeitintervalle zwischen dem Beginn konsequenter schneller Komponenten im vestibulären Nystagmus untersucht Die Kurvenverläufe der Histogramme der Zeitintervalle änderten sich mit der Intensität des Nystagmus analog zu den am optokinetischen Nystagmus berichteten Beobachtungen Während die Intensität des Nystagmus abnahm veränderten sich die Zeitverlaufskurven von anfänglich symmetrisch mono-modalen zu asymmetrisch mono-modalen und schließlich zu multi-modalen Formen in denen die Moden höherer Ordnung ungefähr integrale Vielfache des ursprünglichen Modus

waren Diese Ergebnisse führen zu neuen Hypothesen mit Bezug auf die Nervengeflechte, die den vestibularen und optokinetischen Nystagmus hervorrufen

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Multi-modal interval histograms similar to those described here have been observed in several neural systems (Poggio & Viernstein, 1964; Herz et al., 1964; Bishop et al., 1964). Bishop et al. suggested that multi-modality could occur if a quasi-periodic excitatory action potential process interacted synaptically with a random inhibitory process which intermittently blocked transmission of an excitatory action potential. It will be noted that in such an interaction the output interval histogram would become mono-modal if the mean frequency of the inhibitory process were reduced. It is quite plausible to postulate a similar interaction in the nystagmus generation mechanism, although the single inhibitory and excitatory neurons in the model of Bishop et al. would have to be replaced by groups of neurons, and the output action potential by a burst corresponding to each saccade. Such a model has been found to be capable of simulating observed variations in the interval histogram of vestibular and optokinetic nystagmus (Cheng, 1972; Cheng & Outerbridge, 1973a).

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a cat. As the degree of nasal patency, however, is generally a measure of blood flow in the nasal mucosa (Malm, 1973), changes in patency were used to estimate the sensitivity of the nasal vessels to chemical agents. The responses to intravenously administered drugs in a normally innervated or acutely denervated nose cavity were compared with those in a chronically denervated nose cavity in the same cat. The nasal mucosae in 12 cats were sympathetically denervated by extirpation of one superior cervical ganglion. A parasympathetic denervation was attained in 7 cats by extirpation of one pterygopalatine ganglion by a transpalatal approach. In the latter case, however, sympathetic nerves are also cut. Because of this not only the pterygopalatine ganglion on one side but also the superior cervical ganglia on both sides were extirpated. At the denervations anaesthesia was induced with ether and continued with a short lasting barbiturate, Evipan®-Natrium (Bayer), given intracardially (about 20 mg/kg). Two to eight weeks later the cats were again anaesthetized, now with chloralose 1% (about 80 mg/kg) after induction with ether. The same anaesthesia was given to 6 normal cats. The cats were tracheotomized and the body temperature was kept constant at 38°C by thermostat. A water filled balloon, consisting of a very thin walled latex tube and tied at both ends over a polyethylene tube, was placed in each of the nose cavities (see Malm, 1973). Within the balloon there were small holes in the polyethylene tube, so that the pressure changes in the balloon could be transferred via the polyethylene tube to a transducer connected to an amplifier and a polygraph. Each balloon was connected to a separate transducer and amplifier. When the system was opened the pressure in the balloons was set to 6 cm of water and this resting level was controlled regularly.

Lachrymal secretion was also estimated in the parasympathetically denervated cats using Schirmer's test. Litmus papers were bent over the upper eyelids and a relative measure of the secretion was obtained from the degree of moistening of the papers.

Phenylephrine, noradrenaline, adrenaline and

methacholine were given in physiological saline solutions through a cannula in a femoral vein in doses of 0.0001, 0.001, 0.01, 0.1 and 1 µg/kg. Noradrenaline was given to all the cats. Phenylephrine and adrenaline were given to 5 of the cats which were only sympathectomized and to all the parasympathectomized cats. Methacholine was given to the parasympathectomized cats in order to use Schirmer's test.

RESULT

The records showed both small rapid pressure changes with the same frequency as the heart beats and slower and bigger accompanying the respiration. These and the effect of noradrenaline intravenously are shown in Fig. 1. Phenylephrine, noradrenaline and adrenaline always decreased the amplitude of the small rapid pressure changes and increased the nasal patency. In a few of the cats, where the blood pressure was recorded, the bigger doses of the drugs evoked a blood pressure rise and thus might influence the responses of the drugs on the nose. Blood pressure changes could have been avoided or reduced by giving the agents intra-arterially close to the nose cavities, but as the dosages of the agents then run the risk of being unequal in the two cavities, the intravenous way of administration was chosen.

Comparisons between two normally innervated nose cavities

The changes in nasal patency to chemical agents in two normally innervated nose cavities in the same cat will not necessarily be equal. Anatomical differences may exist and the positions and sizes of the two balloons may vary. Efforts were made to reduce these latter sources of error. The positions were adjusted with the aid of markings on the polyethylene tubes. Two balloons were always chosen with as equal volumes of water as possible at the same pressure. Nevertheless, it was desirable to compare the effects in two normally innervated nose cavities after vasoactive agents. The effects of a series of doses of noradrenaline intravenously were



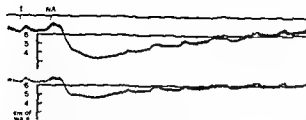


Fig 1 Pressure variations in two water filled balloons, one (upper record) located in a chronically sympathectomized and one (lower record) in a normally innervated nose cavity of a cat. Downward deflection indicates a decreased pressure in a balloon caused by an increased patency. Calibration pressure in cm of water. Time mark in seconds. Noradrenaline $1 \mu\text{g/kg}$ i.v. is given at NA. The start of an inspiration is marked with an I.

The mean differences with S.E.M. between the two sides in 11 cats were for $0.01 \mu\text{g/kg}$ 0.01 ± 0.03 cm of water, for $0.1 \mu\text{g/kg}$ 0.02 ± 0.07 and for $1 \mu\text{g/kg}$ 0.11 ± 0.19 .

Sensitivity after sympathetic denervation

Twelve cats were investigated 2 to 8 weeks after extirpation of the superior cervical ganglion on one side. At first the responses to the drugs in both cavities were recorded with the control side innervated. Then the cervical sympathetic nerve of that side was divided and the responses again recorded. Finally the superior cervical ganglion was extirpated and the effects of the drugs on the acutely and chronically sympathectomized sides compared.

Nine of the cats were given noradrenaline on all three test occasions and the mean values of the pressure changes after different doses are shown in Fig 2. When the control side was normally innervated the mean differences at paired comparisons between the two sides were significant for 1 ($p < 0.001$), for 0.1 ($p < 0.01$) and for $0.01 \mu\text{g/kg}$ ($p < 0.02$) but not for $0.001 \mu\text{g/kg}$ ($p > 0.05$). The threshold doses between the two sides also differed ($p < 0.02$).

After the cervical sympathetic nerve on the control side was acutely cut, the mean responses on the two sides approached one another more closely, but still the mean pressure changes were bigger on the chronically ganglionectomized side. The mean differences were however not signifi-

cant for any of the doses. After acute extirpation of the superior cervical ganglion the mean responses were about the same as after cutting the cervical sympathetic nerve. The mean values of the acutely ganglionectomized sides of the 9 cats, which also are seen in Fig 2, were not significantly different from the values of the chronically denervated ones. Three cats were given noradrenaline only when both sides were sympathectomized, one acutely and one chronically. The differences were not significant when the values from these 3 cats were included.

A supersensitivity may be shown by changes in threshold doses. The mean doses of noradrenaline giving just noticeable responses were smaller in the chronically than in the acutely sympathectomized sides, but the differences at paired comparisons were not significant. The other drugs were given to 5 of the cats and their differences were not significant either, as seen in Table I.

Another sign of supersensitivity may be an increase of the durations of the responses. On the chronically sympathectomized side the pressure responses after noradrenaline seemed to return to the resting pressure later than on the intact (see Fig 1) or the acutely operated side. The mean time to return to resting pressure was significantly longer in the chronically than in the acutely sympathectomized sides (see Table II).

In 4 of the 12 cats the pressure was recorded when the acute preganglionic and postganglionic sympathetic denervations were done. It was easily observed on the record that within a quarter of a minute after the decentralization the pressure increased in the balloon on the acutely operated side and remained on a higher level. No further increase could be seen when the superior cervical ganglion had been extirpated. The pressure on the previously operated side did not change.

Sensitivity after parasympathetic denervation

The sensitivity of the nasal mucosa was investigated in 7 cats 3 to 7 weeks after extirpation of the pterygopalatine ganglion on one side and the superior cervical ganglion on both sides. The

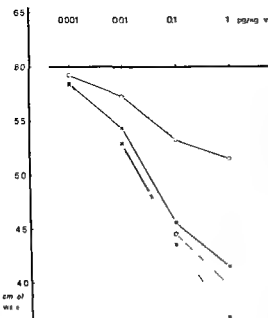


Fig. 2 The mean responses to noradrenaline in normally innervated (O—O) and in chronically sympathectomized nose cavities (O—O) in 9 cats, and in the previously intact cavities after acute sympathectomy (x—x) tested at the same time as the chronically sympathectomized (x—x). Abscissa doses of noradrenaline $\mu\text{g/kg}$. Ordinate pressure in cm of water. The resting pressure was 6 cm of water.

same drugs and doses were given twice and the two sets of balloons, transducers and amplifiers changed place between the tests. This was done in order to reduce the effects of variations in the balloon sizes and positions. The mean of the two tests was taken as a response to a drug. The mean values of the 7 cats were larger for the parasympathetically denervated sides, but paired

Table I The mean threshold doses \pm S.E.M. in $\mu\text{g/kg}$ in acutely and chronically sympathectomized nose cavities

	Phenyl ephrine	Nor adrenaline	Adrenaline
Acutely op side	0.26 \pm 0.18	0.022 \pm 0.011	0.0010 \pm 0.0000
Chronically op side	0.26 \pm 0.19	0.021 \pm 0.011	0.0008 \pm 0.0002
Differences at paired com parisons	$p > 0.05$	$p > 0.05$	$p > 0.05$
No. of cats	5	12	5

Table II The mean durations \pm S.E.M. in seconds of the responses to noradrenaline in acutely and chronically sympathectomized nose cavities

Dose in $\mu\text{g/kg}$	0.01	0.1	1
Acutely op side	36 \pm 7	70 \pm 9	73 \pm 12
Chronically op side	45 \pm 8	85 \pm 9	126 \pm 11
Differences at paired comparisons	$p < 0.01$	$p < 0.05$	$p < 0.001$
No. of cats	11	12	12

comparisons showed that the mean differences between the sides were not significant. The threshold doses and the durations of the responses to higher doses between the sides were not significantly different.

The secretory nerve fibres to the lachrymal gland in the cat originate in or pass through the pterygopalatine ganglion (Botelho et al., 1966). To examine if the lachrymal gland on the transpalatally operated side had developed a supersensitivity, Schurmer's test was used. As evidence of an extirpation of the pterygopalatine ganglion, the litmus papers became more moistened on the parasympathetically denervated side following supraluminal doses of methacholine in all the cats.

DISCUSSION

Malcomson wrote in 1959 "In the sympathectomized nose, the reaction to minute traces of adrenaline intravenously appears earlier than on the intact side." This statement was the starting point for the present study.

To investigate the effects of chronic denervation on the nasal vessels a method using intranasal balloons was chosen as it was simple, gave regular records for many hours and seemed to be as sensitive as the rhinomanometric method used by Malcomson (1959). A disadvantage was that the pressure of the balloons might stimulate afferent nerve fibres. Very little is known, however, of the properties of such nerve fibres in the nasal mucosa. It is possible that there are epithelial receptors which adapt rapidly, just as do receptors in the epipharynx (Nail et al., 1969). Another disadvantage of the method was that

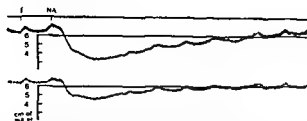


Fig 1 Pressure variations in two water filled balloons one (upper record) located in a chronically sympathectomized and one (lower record) in a normally innervated nose cavity of a cat. Downward deflexion indicates a decreased pressure in a balloon caused by an increased patency. Calibration pressure in cm of water. Time mark in seconds. Noradrenaline $1 \mu\text{g/kg}$ i.v. is given at NA. The start of an inspiration is marked with an I.

The mean differences with S.E.M. between the two sides in 6 cats were for $0.01 \mu\text{g/kg}$ 0.01 ± 0.03 cm of water, for $0.1 \mu\text{g/kg}$ 0.02 ± 0.07 and for $1 \mu\text{g/kg}$ 0.11 ± 0.19 .

Sensitivity after sympathetic denervation

Twelve cats were investigated 2 to 8 weeks after extirpation of the superior cervical ganglion on one side. At first the responses to the drugs in both cavities were recorded with the control side innervated. Then the cervical sympathetic nerve of that side was divided and the responses again recorded. Finally the superior cervical ganglion was extirpated and the effects of the drugs on the acutely and chronically sympathectomized sides compared.

Nine of the cats were given noradrenaline on all three test occasions and the mean values of the pressure changes after different doses are shown in Fig 2. When the control side was normally innervated the mean differences at paired comparisons between the two sides were significant for 1 ($p < 0.001$), for 0.1 ($p < 0.01$) and for $0.01 \mu\text{g/kg}$ ($p < 0.02$) but not for $0.001 \mu\text{g/kg}$ ($p > 0.05$). The threshold doses between the two sides also differed ($p < 0.02$).

After the cervical sympathetic nerve on the control side was acutely cut the mean responses on the two sides approached one another more closely, but still the mean pressure changes were bigger on the chronically ganglionectomized side. The mean differences were, however, not signifi-

cant for any of the doses. After acute extirpation of the superior cervical ganglion the mean responses were about the same as after cutting the cervical sympathetic nerve. The mean values of the acutely ganglionectomized sides of the 9 cats, which also are seen in Fig 2, were not significantly different from the values of the chronically denervated ones. Three cats were given noradrenaline only when both sides were sympathectomized, one acutely and one chronically. The differences were not significant when the values from these 3 cats were included.

A supersensitivity may be shown by changes in threshold doses. The mean doses of noradrenaline giving just noticeable responses were smaller in the chronically than in the acutely sympathectomized sides, but the differences at paired comparisons were not significant. The other drugs were given to 5 of the cats and their differences were not significant either, as seen in Table I.

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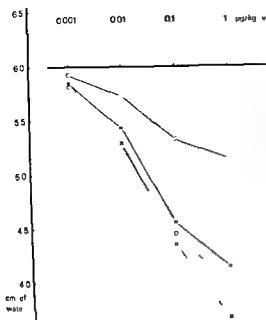


Fig 2 The mean responses to noradrenaline in normally innervated (O—O) and in chronically sympathectomized nose cavities (O—O) in 9 cats and in the previously intact cavities after acute sympathectomy (x—x) tested at the same time as the chronically sympathectomized (x—x). Abscissa doses of noradrenaline in $\mu\text{g/kg}$. Ordinate pressure in cm of water. The resting pressure was 6 cm of water.

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the pressure of the balloons might stimulate afferent nerve fibres. Very little is known, however, of the properties of such nerve fibres in the nasal mucosa. It is possible that there are epithelial receptors which adapt rapidly, just as do receptors in the epipharynx (Nail et al, 1969). Another disadvantage of the method was that

the two balloons in each cavity in a cat could not be exactly equal in size and position. The responses of noradrenaline from two normally innervated nose cavities in the same cat showed, however, that comparisons may be done.

Acute sympathetic denervation decreased the nasal patency probably due to a dilatation of the blood vessels in the mucosa. If the decreased resting tone of the vessels remains for weeks after the denervation this state may be the cause of the bigger responses evoked by noradrenaline on the denervated side compared to the normally innervated. A dose given to dilated vessels may decrease the blood flow more than if the same dose is given to vessels with normal tone. The amount of a drug per unit of time arriving at the smooth muscles of the vessels may increase with increasing blood flow. The responses may also be bigger due to supersensitivity. The part played by the latter cause cannot, however, be determined with the method used.

When the results from a chronically and an acutely denervated side in the same cat are compared, the answer is easier to give. If the tone of the vessels did not change at all during the 2 to 8 weeks after the initial change following extirpation of the sympathetic ganglion the differences of the responses between the acutely and the chronically denervated side must be due solely to supersensitivity. If the tone changed, however, it was certainly in the direction of a regain of tone, i.e. the vessels in the chronically sympathectomized side were less dilated than in the acutely and consequently bigger and longer responses from the former must still be a manifestation of supersensitivity. The differences in the durations of the responses to noradrenaline when a chronically and an acutely sympathectomized side were compared thus seem to be caused by supersensitivity in the chronic side. As the responses following noradrenaline were not significantly bigger in a chronically than in an acutely sympathectomized side a supersensitivity is probably only of minor importance to the differences in responses to the same drug in a chronically denervated compared to a normal side.

The drugs affected the vessels, but they may also evoke secretion. If this secretion is not carried away the nasal patency decreases. The balloons and polyethylene tubes, however, did not obstruct the natural removal of the secretion towards the epipharynx. Furthermore if the nasal glands have about the same sensitivity as the salivary glands (Emmelin & Muren 1951) the doses of the drugs probably evoke no or very little secretion. Recently evidence has been given that stimulation of the vidian nerve evokes a secretion which can be abolished by atropine intravenously and by nicotine applied to the pterygopalatine ganglion (Eccles & Wilson 1973). Thus supersensitivity may develop after extirpation of that ganglion. If the change of sensitivity of the nasal glands to the vasoconstrictor drugs after denervation is about the same as of salivary glands (Emmelin & Muren 1951) it is still unlikely that at least lower doses of the drugs evoke secretion.

The results thus imply that the bigger responses to noradrenaline on the chronically sympathetically denervated nasal vessels compared with the normal vessels are probably only to a minor degree caused by supersensitivity. On the other hand a supersensitivity seems to be the cause of the longer responses on a chronically compared with an acutely sympathectomized mucosa. It may be a sign of only a small change in sensitivity of the nasal vessels that the threshold doses and the magnitude of the responses to supraliminal dosages did not differ between the chronically and the acutely sympathectomized sides. No supersensitivity could be demonstrated in any respect between sides that were both parasympathetically and sympathetically denervated when compared with those that were only sympathetically denervated.

A study of the effects of a preganglionic parasympathetic denervation, which is a part of the denervation done in patients with vasomotor rhinitis was not performed here. The change in sensitivity after such a denervation however ought to be still less than after a postganglionic denervation in accordance with Cannon's law of denervation.

ZUSAMMENFASSUNG

In sympathisch und parasympathisch denervierten Nasenschleimhäuten von 19 Katzen wurde der Effekt von Phenylephrin, Noradrenalin und Adrenalin auf die Blutgefäße mit Hilfe von wassergefüllten Ballons, eingeführt in die Nasenräume, registriert. Einer der Nasenräume diente als Kontrolle in der gleichen Katze. Der Effekt von Noradrenalin war bedeutend grösser auf der Seite, wo 2-8 Wochen zuvor das Ganglion Cervicale Superior entfernt worden war. Diese Unterschiede beruhen aber nicht nur auf Übersensibilität, sondern sind wahrscheinlich auch ein Effekt erweiterter Blutgefäße in der denervierten Nasenschleimhaut. Wenn beide Seiten denerviert wurden, die eine chronisch, die andere akut, waren die Antworten nur dann verschieden, wenn die Dauer verglichen wurde, diese Unterschiede scheinen auf Übersensibilität zu beruhen. Keine bemerkenswerten Unterschiede, weder mit Schwellendosen noch mit Überschwelldosen der Substanzen, konnten gezeigt werden, wenn die postganglionären parasympathischen Nerven durchschnitten worden waren.

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BLOOD GROUP SUBSTANCE A IN CARCINOMAS OF THE LARYNX

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Abstract Tissue from 9 squamous cell carcinomas of the larynx were investigated for the presence of blood group antigen A. The antigen reactivity in the carcinomas was compared, by titration, with the reactivity of adjacent normal epithelium in the same specimen. Normal epithelium reacted positively in all cases except one. In this case the normal epithelium contained areas showing no reactivity for blood group antigen. In 4 out of 9 cases the malignant cells gave completely negative reaction. In 4 specimens there was a patchy distribution of positive reacting cells within the carcinoma. In one specimen the majority of cells in the carcinoma showed no decrease in reactivity.

The demonstration of blood group substances A and B in cells and tissues other than the erythrocytes has a long history (Ladsteiner & Levine, 1926, Kritchewski & Schwartzmann, 1927). In the squamous epithelial cells of the normal vocal cords, their localization on the cell membranes has been demonstrated by use of an immunofluorescence staining technique and the mixed cell agglutination reaction (Szulman, 1960, Kovarik, 1968).

It is well established that many different types of human tumours have tumour associated antigens (Gold & Freedman 1965, Hellström et al, 1971). Furthermore, several studies have indicated that acquisition of a tumour antigen is accompanied by loss of normal cytoplasmic membrane antigens (Burtin et al, 1972).

Partial or complete loss of blood group antigens is reported to occur in premalignant and

malignant diseases developing from tissues in which they are normally present, these include tissue of the bladder (Kay, 1957), oral mucosa (Dabelsteen & Pindborg, 1973), cervix, stomach (Davidsohn et al, 1966), and pancreas (Davidsohn et al 1971), and it has been suggested by some of these authors that the disappearance of antigen is an early sign of malignant transformation.

Kovarik (1968) showed that six of nine carcinomas from the larynx reacted negatively for isoantigen A or B, the rest reacted positively. In normal epithelium A recent publication (Dabelsteen, 1972) states that the amount of blood group antigen A in normal oral mucosa varies from person to person and that sometimes the non-secretor group (Hartmann, 1941) has so little antigen that it can hardly be measured. Any estimation of the reactivity of blood group antigen in carcinomas should therefore be compared with the reactivity in histologically normal mucosa. This was not done in previously published work.

The present paper describes the distribution of blood group antigen in carcinomas of the larynx and compares the reactivity of the blood group antigens in the carcinoma with the reactivity in histologically normal epithelium adjacent to the carcinoma.

MATERIAL AND METHODS

The material consists of surgical specimens from 9 male patients laryngectomized on account of

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Table I Controls for establishing specificity of immunoperoxidase and immunofluorescence staining

Blood group substance	Blood group antisera	Conjugate	Results
A	Phosphate-buffered saline	Labelled antiglobulin	Negative
A	Anti B	Labelled antiglobulin	Negative
A	Anti A absorbed with A ₁ erythrocytes	Labelled antiglobulin	Negative
B	Anti A with known reactivity	Labelled antiglobulin	Negative
A ^a	Anti A with known reactivity	Unlabelled antiglobulin followed by peroxidase reaction	Negative
A	Anti A with known reactivity	Labelled antiglobulin	Positive

^a For immunoperoxidase stainings only

squamous cell carcinomas of the larynx. All patients belonged to blood group A. In all specimens histologically normal mucosa was found adjacent to the tumours. Before surgery all carcinomas had been treated by irradiation.

Biopsies of five of the carcinomas taken before irradiation were also investigated. Histologically normal mucosa was present in four of these biopsies.

The surgical specimens as well as the biopsies were fixed in 10% formalin, embedded in paraffin and sectioned at 5 μ m. Blood group substances were detected in the epithelium by two different immunological staining procedures, the immunoperoxidase (IP) method (Avrameas, 1970) and the immunofluorescence (IF) method (Weller & Coons, 1954; Dabelsteen & Fulving, 1971).

Both staining methods were used as double-layer techniques, the first layer being a human blood group test serum and the second layer a rabbit antihuman IgG globulin conjugated with peroxidase for the IP staining and with fluorescein isothiocyanate (FITC) for the IF staining. The blood group antiserum was provided by Statens Seruminstitut, Copenhagen and the conjugates were purchased heavy chain specific from Dakopatts A-S, Copenhagen.

The peroxidase conjugate had an antihuman IgG titre of 300 (i.e. 1 ml conjugate absorbs 300

μ g pure IgG). The molar peroxidase/protein ratio was 1:20. A working titre of 1:20 was found by chessboard titration.

The FITC conjugate had an antihuman IgG titre of 200. The mean optical density ratio 495/280 nm was 0.66 with 0.30 as lowest and 0.95 as highest ratio. (Unconjugated immunoglobulin molecules with an O.D. ratio below 0.3, and conjugated immunoglobulins with an O.D. ratio above 0.95, were removed by ion exchange chromatography.) By chessboard titration (Wick & Beutner, 1970) a working titre at 1:40 was found. To ensure that the IF and IP stainings were specific, control reactions were made. These are summarized in Table I.

The fluorescence microscope used was a Leitz Orthoplan with a darkfield oil immersion condenser. The primary filter was a FITC-interference filter with red contrast band (Rygaard & Olsen, 1969). The secondary filter was a 2 mm glass filter (Schott & Gen, Germany) matched to fit the primary filter (Rygaard & Olsen, 1971).

The amount of blood group antigen in the epithelium was estimated by a two-fold serial dilution titration, and the reciprocal of the highest dilution giving positive reaction was regarded as the endpoint titre. The reactions were read as positive or negative.

The surgical specimens were all titrated in the IP staining. The biopsies and the corresponding

surgical specimens were stained simultaneously with the IF staining

RESULTS

In all 5 cases where it was possible to compare irradiated tissue with tissue from the same patient before irradiation, no difference in reactivity for blood group antigen was found. Furthermore, although the end-point titres are different in the IP and the IF staining reactions, the reaction patterns are identical (Table II).

Blood Group Antigen Reactivity in Laryngeal Carcinomas and Adjacent Normal Epithelium (IP Stainings)

Normal epithelium

In all cases except one the normal mucosa reacted positively for blood group antigen A (Table III). The positive reaction was seen as a uniform brown staining of the cell membranes in the spinous cell layer, the basal cell layer and the superficial cell layers do not react (Fig. 1).

In one case (no. III) the normal epithelium contained areas showing no reactivity for blood group antigen (Fig. 1).

Squamous cell carcinoma

Three different patterns of reaction were seen in carcinomas.

(1) In 4 of the 9 cases the squamous cell carcinoma

Table II End point titres obtained in immunofluorescence staining of non irradiated and irradiated laryngeal mucosa

— negative
n.t. = not tested
The numbers in Tables II and III refer to the same patients

Patient no	Biopsy non irradiated		Surgical specimens irradiated	
	Normal	Tumour	Normal	Tumour
I	1:32		1:32	
II	n.t.		1:4	
III	1:32	1:8	1:32	1:8
IV	n.t.	1:32	Conc	1:32
V	1:8		1:8	

Table III End-point titres obtained by staining normal epithelium and laryngeal carcinomas in an immunoperoxidase staining method

— negative
n.t. = not tested
The numbers in Tables II and III refer to the same patients

Patient no	Normal epithelium	Tumour	
		Most cells	Patchy areas
I	1:16	—	—
II	Conc	—	—
III	1:8	—	Conc
IV	Conc	1:16	—
V	1:4	—	—
VI	1:8	—	1:8
VII	Conc	—	Conc
VIII	1:4	—	1:8
IX	Conc	—	—

reacted negatively with all concentrations of anti-A serum (Table III, Fig. 2).

(2) In 4 cases about one-third of the cells in the carcinoma reacted positively for blood group antigen. The positive cells were arranged in small groups unevenly distributed within the carcinoma (Fig. 3). The positive cells in three of these cases had endpoint titres of the same magnitude as the normal adjacent epithelium. In one case (no. III) the end-point titre of the cancer cells was lower than that of the adjacent normal epithelial cells.

(3) In 1 case (no. IV) all the tumour cells reacted positively with end-point titres which were higher than those obtained in normal adjacent epithelium (Fig. 4).

Finally, in 2 cases where epithelium adjacent to the carcinoma exhibited slight dysplasia no change in blood group antigen was noted.

DISCUSSION

The present work evaluates the amount of blood group antigen A in 9 squamous cell carcinomas of the larynx by making comparison with the amount of antigen in normal mucosa of the same patient. It shows that in 4 of the cases there was a total loss of blood group antigen

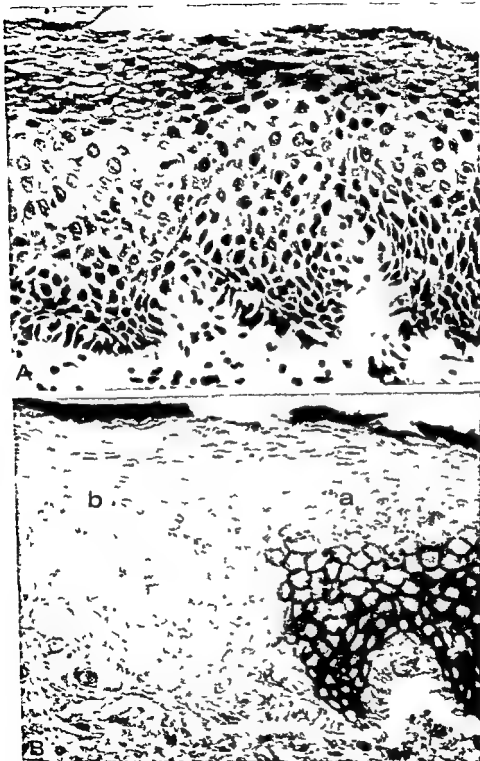


Fig 1 Normal squamous epithelium adjacent to laryngeal carcinoma (A) Haematoxylin and eosin staining (B) Immunoperoxidase staining of neighbouring section

Black intercellular spaces indicate positive reacting cells (a) normal distribution of blood group antigen (b) loss of antigen $\times 200$

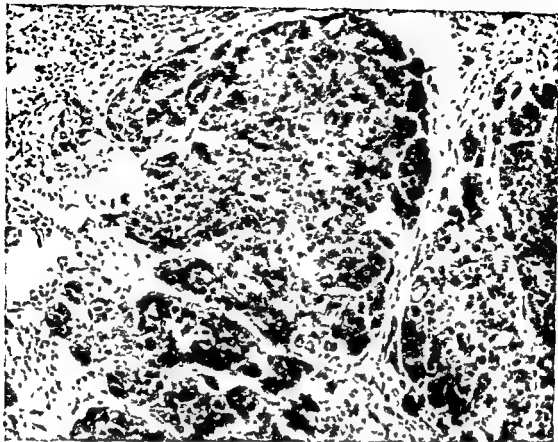


Fig 2 Squamous cell carcinoma of the larynx. Immunoperoxidase staining followed by haematoxylin and eosin

staining. All cancer cells react negatively for blood group antigen. $\times 77$

the carcinomas. In 4 other cases there was a decrease in reactivity of blood group antigen illustrated by the patchy distribution of positive cells. In 1 case (no IV) there was no decrease of blood group antigen, on the contrary, this case demonstrated a stronger reaction in the carcinoma than in the adjacent mucosa.

The findings in the present study are in agreement with the previous study of A and B antigen in carcinomas of the oral mucosa. However, the titration of anti-A serum, which has proven useful in previous work (Dabelsteen & Pindborg, 1973) is of value in only one of the present series. In this case the normal epithelium adjacent to the tumour and the tumour cells both reacted positively with the higher concentrations of antibody but only the normal cells showed reactivity for blood group antigen A when the anti-A serum was diluted.

It is of interest to notice that 2 cases exhibiting epithelial dysplasia adjacent to the cancer showed no decrease in blood group antigen A reactivity. This is in contrast to previous studies which have shown a decrease in quantity of blood group antigen in lesions exhibiting epithelial dysplasia, including lesions of the oral mucosa (Dabelsteen & Fulling, 1971) and the mucosa of the uterine cervix (Davidsohn et al., 1969). As some, but not all, lesions exhibiting epithelial dysplasia develop into cancer, it would be of great interest to know how the change in blood group antigen reactivity is correlated to the prognosis of the lesions.

The relatively large number of cases containing patchily distributed areas of cancer cells reacting positively for blood group antigen A indicates that the change in reactivity of A antigen is a tumour-associated chance rather

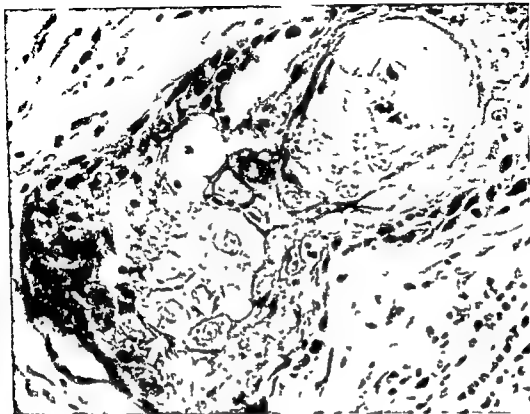


Fig 3 Squamous cell carcinoma of the larynx. Immunoperoxidase staining followed by haematoxylin and eosin staining. Area showing patchy distribution of blood

group antigen. Black intercellular spaces (arrow) indicate presence of blood group antigen $\times 250$

than tumour specific. This of course decreases the diagnostic value of change in blood group antigen reactivity for laryngeal epithelium with suspected malignant properties. No attempt has in the present study been made to correlate the presence or absence of staining with the biological behaviour of the tumour. This seems a worthwhile investigation to continue with. It has been mentioned (Davidsohn 1972) that no other disease is presently known in which the isoantigens A and H are lost to the same extent and with the same regularity as in carcinomas, this problem needs very careful investigation before the diagnostic value of decrease in epithelial blood group antigen can be properly evaluated.

Several studies have demonstrated antigenic changes of the cell membrane during malignant transformation. These changes include appear-

ance of new antigens as well as loss of normal cytoplasmic membrane antigen. Whether the loss of blood group antigen in carcinomas of the larynx is connected with appearance of new antigen is unknown. Furthermore it would be of interest to know whether the positive tumour cells are really positive for blood group antigen or whether a cross reactivity takes place between blood group antigen A and a tumour antigen. That this is possible has recently been demonstrated by Gold et al (1972).

In conclusion the present study has shown that the number of blood group antigen A positive cells is appreciably reduced in most laryngeal carcinomas. Furthermore it has shown that this is a tumour associated change and not a tumour-specific change.



Fig 4 Squamous cell carcinoma of the larynx (A) Haematoxylin and eosin staining (B) Immunoperoxidase staining of neighbouring section. Black intercellular spaces indicate positive reacting cells. Note that nearly all cancer cells react positively.

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ZUSAMMENFASSUNG

Gewebe von 9 Larynx Plattenepithelkarzinomen wurden auf das Vorkommen von Blutgruppe A Antigen hin untersucht. Die Antigen Reaktionsfähigkeit in den Karzinomen wurde mittels Titrieren mit der Reaktionsfähigkeit angrenzenden Normalepithels bei demselben Präparat verglichen. Das normale Epithel reagierte mit einer

von positiv reagierenden Zellen im Karzinom. In einem Fall zeigte ein Mehrzahl der Zellen im Karzinom keine Verminderung der Reaktionsfähigkeit.

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THE EFFECTIVENESS OF A STERNOMASTOID MUSCLE FLAP IN PREVENTING POST-PAROTIDECTOMY OCCURRENCE OF THE FREY SYNDROME

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(Received June 28 1973)

Abstract Among over 350 parotidectomies the effectiveness of a pedicled sternomastoid muscle flap in preventing post parotidectomy occurrence of the Frey Syndrome was tested during the past year in a random sampling of 35 patients with such a muscle flap and 35 control patients. Results of the study indicate that the muscle flap, although improving cosmetic defects associated with surgery is incapable of inhibiting the Frey Syndrome and that other means must yet be devised to prevent the occurrence of gustatory sweating following parotid surgery.

The Frey, or auriculotemporal, Syndrome is an unusual clinical problem which involves facial flushing or sweating following parotid gland infection surgery or trauma. Several theories have been put forward concerning the mechanism by which this syndrome occurs, but the most plausible explanation appears to be misdirection of parasympathetic secretomotor fibres from the auriculotemporal nerve (which under normal circumstances provides secretomotor fibres to the parotid gland) to sweat glands or small vessels overlying the site of the gland (Blumenfeld & Friedman, 1967, Smith et al, 1970). Subsequent stimulation of the nerve results in sweating or flushing of the face. The complication following surgery of the parotid gland has been estimated to be 25-97% (Hunt et al, 1966) although most patients do not require medical attention.

Since 1964, over 350 parotidectomies have been performed at the ENT Clinic of the University of Göttingen. A pedicled flap from the sternomastoid muscle was utilized in se-

lected patients in order to prevent occurrence of the Frey Syndrome following parotid surgery. It was thought that this muscle flap would act as a "barrier" to inhibit the misdirection of the auriculotemporal fibres subsequent to surgery. The present study was a critical analysis of the effectiveness of this sternomastoid muscle flap in preventing post-parotidectomy occurrence of the Frey Syndrome.

MATERIALS AND METHODS

As previously noted, a sternomastoid muscle flap was utilized to cover the operative defect in selected patients undergoing parotid surgery for non malignant disease. This muscle flap was based superiorly, and originated from the insertion of the sternomastoid muscle on the mastoid process (Fig. 1). A thick muscle pedicle could then be sutured over the main trunk and primary rami of the facial nerve to cover the site of emergence of the auriculotemporal nerve into the retromandibular fossa.

A random sampling of 35 patients with a sternomastoid muscle flap as described and a control group of 35 patients without such a flap were examined for incidence and severity of the Frey Syndrome following surgery.

Of the patients studied, parotid surgery had been performed between 1 and 6 years previously. The essential postoperative course of these patients had been without significant consequent

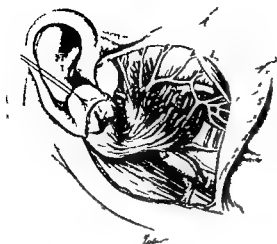


Fig 1 Pedicled sternomastoid muscle flap used to cover operative defect in patients undergoing parotid surgery

A profile of all patients studied is given in Table I

All patients were questioned concerning subjective symptoms related to the Frey Syndrome. These questions involved asking whether or not the patients noticed facial warmth, flushing or sweating in association with such gustatory stimuli as eating, smoking or use of cholinergic medications. The Minor starch iodine test was then performed, as described by Laage-Hellman (1957). An alcohol iodine oil solution was prepared by mixing 3 grams of iodine with 20 grams of castor oil, and bringing the final solution to 200 grams by the addition of absolute alcohol. Both lateral portions of each patient's face and upper regions of the neck were painted with this preparation, and the solution allowed to dry. These areas so prepared were then lightly covered with starch powder. Lemon slices were employed as stimulus to provoke gustatory sweating. Patients were required to chew and suck these lemon slices for approximately 2 minutes. As noted by Laage-Hellman (1957) the Minor test is capable of producing objective documentation of even unappreciable sweating. Any sweating whatsoever produces a blue dis-

coloration of the iodine starch mixture which varies in direct proportion to the severity of the sweating. Minimal secretion of sweat results in coloration of the openings of the individual sweat glands, as marked by tiny blue punctae. More pronounced sweating produces varying confluent areas of blue staining. Documentation is accomplished by photography, and the degree of the Frey Syndrome when occurring can be categorized as slight, moderate or severe (Fig 2).

RESULTS

A composite of all patients studied (Table I) shows that the majority of patients (42/70, 60%) underwent lateral parotidectomy as treatment for benign tumour. In testing patients for incidence of the Frey Syndrome it soon became apparent that pathological diagnosis of the excised parotid gland had no significance in the occurrence of the syndrome. Consequently, Tables II and III indicate the incidence and severity of the Frey Syndrome in patients with or without sternomastoid muscle flaps following

Table I Patients studied in determining the effectiveness of sternomastoid muscle flaps in preventing postoperative occurrence of the Frey Syndrome

Years following surgery	No of patients		Histological diagnosis	
	Musc flap	Control group	Chronic parotitis	Benign tumour
1 Lateral parotidectomy				
1	4	1	0	5
2	5	8	3	10
3	6	5	0	11
4	3	8	2	9
5	3	0	1	2
6	2	5	2	5
2 Subtotal/total parotidectomy				
1	2	1	1	2
2	3	3	1	5
3	1	1	2	2
4	3	2	1	4
5	1	0	0	1
6	2	1	0	3
	35	35	11	59

THE EFFECTIVENESS OF A STERNOMASTOID MUSCLE FLAP IN PREVENTING POST-PAROTIDECTOMY OCCURRENCE OF THE FREY SYNDROME

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Of the patients studied, parotid surgery had been performed between 1 and 6 years previously. The essential postoperative course of these patients had been without significant consequent

Table II Incidence of Frey Syndrome subsequent to lateral parotidectomy

Years following surgery	No of pats	Severity	Muscle flap			Controls		
			Subjective complaints	Minor Test		Subjective complaints	Minor Test	
				Pos	Neg		Pos	Neg.
1	5	Slight	0	3	0	0	1	0
		Moderate	1	1	0	0	0	0
		Severe	0	0	0	0	0	0
2	13	Slight	2	2	0	0	3	0
		Moderate	1	1	0	0	2	0
		Severe	2	2	0	1	3	0
3	11	Slight	1	4	1	1	5	0
		Moderate	0	1	0	0	0	0
		Severe	0	0	0	0	0	0
4	11	Slight	0	0	0	2	5	1
		Moderate	1	2	0	1	1	0
		Severe	1	1	0	1	1	0
5	3	Slight	0	1	0	0	0	0
		Moderate	0	0	0	0	0	0
		Severe	2	2	0	0	0	0
6	7	Slight	1	1	0	1	3	1
		Moderate	0	1	0	0	1	0
		Severe	0	0	0	0	0	0
Totals	50		12	22	1	7	25	2

Table III Incidence of Frey Syndrome subsequent to subtotal or total parotidectomy

Years following surgery	No of pats	Severity	Muscle flap			Controls		
			Subjective complaints	Minor Test		Subjective complaints	Minor Test	
				Pos	Neg		Pos	Neg
1	3	Slight	0	1	0	0	0	0
		Moderate	0	0	0	1	1	0
		Severe	0	1	0	0	0	0
2	6	Slight	0	3	0	0	3	0
		Moderate	0	0	0	0	0	0
		Severe	0	0	0	0	0	0
3	2	Slight	0	1	0	0	1	0
		Moderate	0	0	0	0	0	0
		Severe	0	0	0	0	0	0
4	5	Slight	0	1	0	0	0	0
		Moderate	0	1	0	0	2	0
		Severe	0	1	0	0	0	0
5	1	Slight	0	0	0	0	0	0
		Moderate	1	1	0	0	0	0
		Severe	0	0	0	0	0	0
6	3	Slight	0	0	0	0	0	0
		Moderate	2	2	0	0	1	0
		Severe	0	0	0	0	0	0
Totals	20		3	12	0	1	8	0

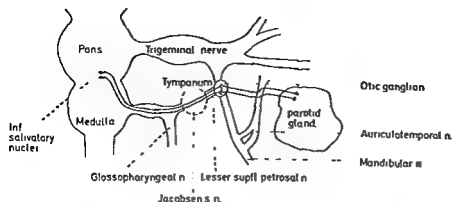


Fig 3 Normal neural pathway for parotid secretomotor stimulation

DISCUSSION

The present study attempted to define the effectiveness of a sternomastoid muscle flap in preventing post-parotidectomy occurrence of the Frey Syndrome. However, positive documentation for varying degrees of the syndrome was found in almost all patients studied (91.5–100%), indicating ineffectiveness of the flap in inhibiting gustatory sweating following parotid surgery.

Fig 3 shows a schematic representation of the normal neural pathway for parotid secretomotor stimulation. Preganglionic parasympathetic fibres initiate in the inferior salivatory nuclei within the lower pons. These fibres then pass with the glossopharyngeal nerve from the brainstem, and enter the tympanic cavity through a canaliculus to form the tympanic or Jacobson's nerve. The fibres proceed across the promontory of the middle ear between the round window and Eustachian tube orifice. The fibres next become part of the tympanic plexus which overlies the promontory, and exit from the middle ear through the deep or lesser superficial petrosal nerve. The preganglionic fibres further pass with this nerve to the otic ganglion, at which site synapses occur with postganglionic neurones. These postganglionic fibres leave the ganglion, and continue with the auriculotemporal nerve to the parotid gland. As previously noted, disruption of the terminal fibres of the auriculotemporal nerve in some manner produces misdirection of the fibres to skin vessels and sweat glands. These endorgans subsequently respond to gusta-

tory stimulation by vasodilation or secretion of sweat.

The prevalence of the Frey Syndrome following parotid surgery has previously been well documented by Laage Hellman (1957), and was corroborated by the present study. Under most circumstances, patients with the syndrome remain clinically asymptomatic or do well with supportive measures alone. This was evident during the present study in that no patient with subjective or objective evidence for the syndrome considered clinical aid necessary.

Varied measures have been employed to manage the Frey Syndrome in those patients with significant distress. These modalities have included the use of topical or systemic anticholinergic medications, radiotherapy to ablate involved sweat glands or fibrose surface vessels, excision of skin, removal of the otic ganglion, section of the auriculotemporal nerve, intracranial division of the glossopharyngeal nerve, as well as intratympanic section of the tympanic and chorda tympani nerves (Laage Hellman 1957, Golding Wood, 1962, Hunt et al 1966, Blumenfeld & Friedman, 1967, Smith et al 1970). However, no treatment modality has met with absolute success over prolonged periods of time, and patients invariably learn to tolerate their discomfort.

It was intended that a sternomastoid muscle flap used to cover the operative defect following parotidectomy would serve as a barrier between terminal fibrils of the auriculotemporal nerve and skin vessels and sweat glands, thus providing

prophylaxis against possible occurrence of the Frey Syndrome. This was not realized by the present study.

The sternomastoid muscle flap does have usefulness in minimizing a cosmetic defect resulting from parotid surgery (Jost et al., 1968). However, it does not inhibit gustatory sweating subsequent to surgery, and other means must yet be devised to prevent occurrence of the syndrome following parotid surgery.

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Grateful acknowledgment is made of Hans Gunther Bräkefeld for medical photography and Ursula Krüger for illustrations provided during this investigation.

ZUSAMMENFASSUNG

Unter ca. 350 Parotidektomierten der letzten Jahre wurde eine repräsentative Stichprobe von 35 Patienten ausgewählt. Bei der I. Gruppe wurde ein Schwenklappen des M. sternocleidomastoideus gebildet, um das Auftreten des Frey'schen Syndroms nach der Parotidektomie zu verhindern. Diese Gruppe wurde mit einer Kontrollgruppe von 35 Patienten verglichen, bei denen dieser Schwenklappen nicht benutzt wurde. Es zeigt sich, dass

mit dem Muskelschwenklappen eine bessere kosmetische Deckung des chirurgischen Defektes erzielt werden konnte. Das Frey'sche Syndrom blieb jedoch unbeeinflusst. Es erscheint daher notwendig, nach anderen Massnahmen zur Verhinderung des gustatorischen Schwitzens zu suchen.

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GIANT CELL TUMORS OF BONE IN THE ENT ORGANS

Report of Two Cases in the Frontal Sinus

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Abstract This is a report on two patients with giant cell tumors of bone located in the frontal sinus. In both patients potential malignancy was suspected. The first patient, female, had a polyostotic form of the disease located in the frontal sinus and acromion. The second patient, male, had the cystic form of tumor in the frontal sinus, with involvement of the ethmoidal labyrinth. The laboratory findings of both patients showed no abnormalities. Both patients were treated surgically and showed no recurrence 2½ years after operation. The authors discuss the cases collected from the otolaryngological literature with occurrence in various organs of the ENT region. They are very rare in this area and there is therefore little experience of their diagnosis and treatment. As the diagnosis of this disease in the ENT organs is made mostly after the operation, the postoperative biopsy is of utmost importance as to the prognosis. Long term observation of patients is imperative. Primary irradiation is not recommended.

Giant cell tumors of bone are very rare in the ENT organs. The diagnosis, prognosis and treatment are of great importance. In the last two years two cases of giant cell tumors with an extremely rare localization in the frontal sinus were observed at the ENT clinic of the Faculty of Hygiene in Prague.

CASE REPORTS

Case 1

Female (M No protocol 9176) born 1908, noticed a painless growth in the frontal region within the last 3 months. At the time of admission to the clinic a painless swelling of hard consistency, 3 × 3 × 1½ cm in size, was found. Other findings in the ENT region were negative, and no lymph nodes were palpable. X-ray

(Dr Blaha) revealed a semicircular homogeneous shadow projecting into the upper part of the frontal sinus (Fig 1). The tumor was excised together with the periosteum and adjacent bone. It was of chestnut size, filling the upper part of the left frontal cavity. The posterior wall of the sinus was eroded up to the thickened dura mater, with a bony defect 2½ × 2½ cm in size. The mucous membrane of the sinus was thickened without granulations or secretion.

Fig 2 shows the postoperative appearance of the patient. The histological report (Dr Stolz) describes a highly vascular tumor invading and destroying the bone, and containing numerous giant cells. The tumor showed many areas of fibrosis with apparent collagen formation. In some places capillaries predominated, and focal hemorrhages were also noted. The bone marrow showed many giant cells of blastomatous type (Fig 3). During a later examination of the whole skeleton X-ray revealed a further focus in the acromion (Fig 4). Laboratory data showed serum calcium 11.4 mg ionised calcium 4.1 mg %, and normal values for phosphorus and for alkaline and acid phosphatases. The postoperative course was without complications, and the patient is free from recurrence after 2½ years.

Case 2

I H male, born in 1949 (No protocol 6028). Six months prior to admission he observed the left exophthalmus increasing. A swelling in the



Fig 1 X ray showing the tumor of the frontal bone and opacity of the frontal sinus

roof of the orbita was found, occupying the floor of the left frontal sinus (Fig 5). The left eye was depressed downward though without impairment of movement or sight. X ray (Dr Blaha) revealed a light diffuse opacity of the left frontal sinus (Fig 6). On tomography the major part of the left frontal sinus was occupied by a tumor of ovoid shape. There was a slight opacity in the ethmoidal cells. Evidently a part of the roof and of the internal ridge of the left orbit, and the posterior wall of the sinus were destroyed. Serum calcium, phosphorus alkaline and acid phosphatases were normal. During the operation performed through a Killian skin incision a defect in the inferior wall of the frontal sinus, $2\frac{1}{2} \times 2\frac{1}{2}$ cm in size, was detected. The soft tumor masses prolapsed through the defect and completely filled the widely opened sinus. The tumor was dark brown and soft with a solid portion in the lateral recess covered

with a film of pus. After removal of granulations, a pulsating cyst the size of a hen's egg and containing 15 ml of pink fluid was discovered.

The cyst had eroded the posterior wall of the sinus, thus forming a bony defect, 4×4 cm in size, and adherent to the dura mater. Tumor tissue involved the ethmoidal labyrinth. It was exenterated and the tissue was found to have the same appearance as the tumor in the frontal sinus. A wide drainage into the nose was performed. Normal healing occurred after 2 weeks. The patient is now in good health $2\frac{1}{2}$ years after operation (Fig 7).

The histologic finding was as follows. The section (Fig 8) shows an invasive osteogenic tumor arising near the periosteum, containing numerous spindle and round cells arranged around branched capillaries. Formation of osteoid and mature bone is noticed in some areas. Numerous giant cells are found. The capillaries are enormously dilated, even to the formation of cavernous spaces. The tumor fills the frontal sinus leaving portions of the mucosal lining intact.



Fig 2 The patient after operation

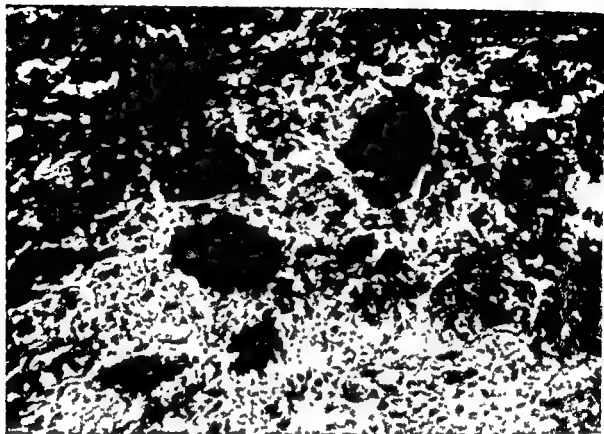


Fig 3 Histologic picture of tumor tissue. Note many giant cells (hematoxylin-eosin $\times 650$)

DISCUSSION

Taffe (1964) classifies giant cell tumors into 3 groups: (1) giant cell tumors of bone, (2) reparative granuloma, (3) giant cell tumors of hyperparathyroidism. Zollinger (1968) divides so-called brown tumors into 3 major groups: (1) osteitis fibrosa Recklinghausen, (2) juvenile bone cysts, (3) osteoclastomas. Doerr (1970) calls this group of bone tumors fibromas, which he divides into pure fibromas, solitary bone cysts, and benign giant cell bone tumors. Clinically and microscopically there exists considerable confusion as to what constitutes a giant cell tumor. Only recent studies using ultramicroscopic methods have brought some clarity in this question. These tumors are characterized by the presence of masses of mononuclear cells which are ovoid or spindle-shaped with a small amount of cytoplasm. The nuclear

membrane is smooth and thin. In this mass of small cells, large multinuclear giant cells are embedded. They contain up to 80 nuclei, as found by Steiner & Ghosh (1972) in their microscopic studies. The paper of Hideya et al (1970) who studied the ultrastructure of these tumors suggests that the giant cells are formed by fusion of round stroma cells. These authors also point out that the fine structure of the giant cells is indistinguishable from osteoclasts according to Morton (1956). Stuart called these tumors actually osteoclastomas. Steiner & Ghosh (1972) on the basis of ultramicroscopic studies, think that these giant cell tumors originate from undifferentiated connective tissue of bone marrow and giant cells by fusion of mononuclear cells. They differ from osteoclasts in that they do not appear to participate in active bone resorption. The mononuclear cells rather than giant cells, are the principal cells



Fig 4 Focus of the giant cell tumor in the acromion



Fig 6 X ray showing opacity of the frontal sinus and ethmoidal cells



Fig 5 The patient before operation. Note the swelling of the floor of the left frontal sinus



Fig 7 The patient after operation

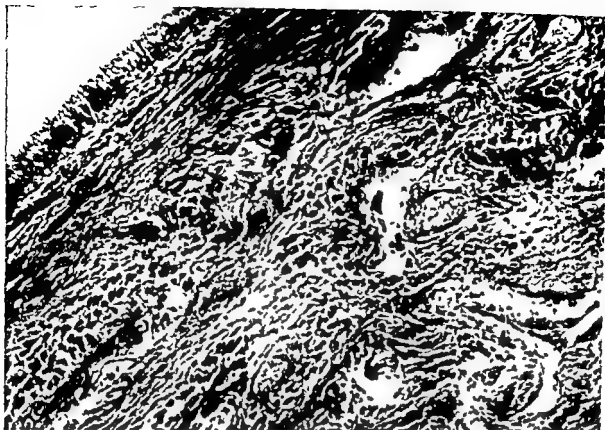


Fig 8 Histologic picture of the tumor. Note the preserved epithelial lining of the frontal sinus (hematoxylin-eosin $\times 250$)

in this tumor. In many of them areas of collagen deposits and spicules of bone are noted. According to Jaffe the hemorrhage described in many of these tumors is a differential point. When present the brown tumor of hyperparathyroidism should be suspected. Crowding of the stromal cells and a decrease in the number of giant cells, as well as an increase in the number of mitoses is considered a sign of greater aggressive potential. According to Murphy & Ackerman (1956) approximately 10% of all giant cell tumors are malignant at the time of first examination.

As far as the origin of giant cell tumors is concerned, Geschickter & Copeland (1936) suggest that they arise from abnormal hyperplasia of osteoclasts beside areas of enchondral ossification. On the other hand Jaffe (1964) thinks they originate from undifferentiated supporting connective tissue of the bone marrow.

As with many other bone tumors trauma or congenital origins have been suspected as etiologic agents, though without proof.

Incidence

Giant cell tumors occur mostly in long bones of the skeleton. The frequency of their occurrence in the maxillary cavity, mandible and alveolar process is approximately 9% (Švejda, 1968). Hlavacek (1931) published an earlier report of 3 patients with localization in the mandible. These tumors are rare in the ENT organs. They occur mostly in the maxillary cavity (Kutvirt, 1926, Geschickter & Copeland, 1936, Mela & Mongini, 1966). Localization in the frontal bone has been reported by Horníček (1948) and by Gignoux et al (1957). Nasal bones were involved in the case of Sgouras (1971). The sphenoidal sinus may be site of

giant cell tumor as well, as reported by Geisinger et al (1970) and by Gary et al (1970) Emley (1971) collected 14 cases in this site from the world literature. I have not found any case in the literature describing giant cell tumor with a large cyst in the frontal sinus, as was the case in one of our patients. The region of the temporal bones is also sometimes the site of occurrence of these tumors but very rarely. The squamous temporal bone and mastoid process were involved in the cases of Kutvirt (1926), Horníček (1948), Ježek (1951) and Sakamoto (1970). The middle ear was involved in the cases of Rosenwasser (1969) and Gignoux (1970). Hall (1972) reported the very rare occurrence of a giant cell tumor in the larynx.

The course of the disease is slow and without appreciable pain if the tumor is localized in the ENT organs. Malignant variants are rarely observed but local recurrences are frequent.

Diagnosis

Diagnosis rests on physical and laboratory examination, X ray, and careful clinical survey. If sufficient tissue is available, biopsy is most important for diagnosis (Morton 1956). Diagnosis from biopsy rests on a careful evaluation of both the stroma and the giant cells. Many bony lesions have giant cells. The lesion most likely to be confused with giant cell tumors such as the so-called brown tumor of hyperparathyroidism. In the latter lesion giant cells are less numerous, unevenly distributed and often surrounded by areas of haemorrhage. Serum calcium, phosphorus and alkaline and acid phosphatase determinations are invaluable aids to making the correct diagnosis. While making the diagnosis one has to take into consideration the fact that giant cells are present in many other lesions of bone: in benign chondroblastomas, non-osteogenic fibromas, aneurysmal bone cysts, simple bone cysts with cellular lining, reparative granuloma, lesions of hyperparathyroidism and osteogenic sarcoma. All these must be considered in the differential diagnosis, as stated by Dahling et al (1962).

Treatment

Treatment consists mainly of surgical procedure, i.e. excochleation and if the tumor is strictly delimited, in complete extirpation of the tumor, together with adjacent bone. Primary irradiation is contra-indicated since the tumor may undergo malignant changes. As histological examination cannot altogether exclude malignancy, long term observation of the patient is necessary.

RESUME

Rapport sur deux malades atteints de tumeurs à cellules géantes localisées dans le sinus frontal. La première maladie était atteinte d'une polyostéotique formée avec la localisation de la tumeur dans le sinus frontal et l'acromion. Le deuxième patient homme avait une cystique formée de la tumeur dans le sinus frontal avec l'envahissement du labyrinthe ethmoïdal. Le bilan humoral de ces malades était normal. Les malades étaient traités chirurgicalement et sont 2½ années sans récurrence.

ZUSAMMENFASSUNG

Ein Bericht über zwei Kranken die mit gigantischen Geschwulsten der Stirnhöhle betroffen waren. Die erste Patientin wies eine polyostotische Form aus. Die Geschwulst wurde in der Stirnhöhle und Acromion lokalisiert. Der zweite Kranke hatte eine zystische Form der Geschwulst in der Stirnhöhle. Die geschwulstartigen Veränderungen breiteten sich auch im ethmoidalen Labyrinth aus. Die laboratorischen humoralen Befunde wiesen bei beiden Kranken keine Abnormalität auf. Beide wurden chirurgisch behandelt und sind 2½ Jahre ohne Rezidive.

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THE VASCULAR ANATOMY OF THE RHESUS MONKEY COCHLEA

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Abstract The vascular pattern of the Rhesus monkey cochlea was demonstrated by the aid of contrast injection. The capillary areas are very similar to the human, rabbit, and guinea pig cochlea. One significant exception is the infrequent occurrence of a vessel under the tunnel of Corti. Further, the capillary areas of the external wall at the basal end of the cochlea are more well formed than in the other mammals. The course and ramification of the vessels in the modiolus is more irregular than in the rabbit and guinea pig cochlea and more similar to the human cochlea. The irregular occurrence of the vessel under the outer hair cells indicates that the Rhesus monkey is a particularly interesting animal for cochlear research.

The cochlear vascular anatomy of those mammals which are most commonly assessed by experimental otologic research is in general well known (see Axelsson, 1968). However, the vascular anatomy of the Rhesus monkey cochlea has not been reported and in general there are only sparse reports concerning the cochlear vascular anatomy of any monkeys. The importance of investigating the Rhesus monkey is based primarily upon the increasing use of this mammal for experimental cochlear research and the presumed similarity between monkey and man in regard to both cochlear anatomy and function.

The earliest description of the vascular anatomy in the monkey (*Macacus*) was given by Nabeya (1923). The most remarkable findings in the *Macacus* were one spiral vessel under the

tunnel of Corti and two zones of capillary network: one in the spiral limbus and one in the tympanic lip. There were individual variations in the gross venous drainage from the cochlea between animals. Nabeya concludes that all capillaries of the labyrinth of the monkey closely resemble those of the human ear.

Hawkins & Johnsson (1968) reported a large outer spiral vessel in the Rhesus monkey fetus. In a later fetal stage an inner spiral vessel could be observed simultaneously to the outer spiral vessel. These authors demonstrated two spiral vessels in the spiral lamina of the squirrel monkey. Johnsson (1972) reports an incomplete outer spiral vessel or none at all, the vessel being best developed in the basal turn in different monkeys, i.e. pigtail, crab-eating, stump-tail Rhesus and squirrel monkey.

The purpose of this study is to extend the sparse current knowledge of the vasculature of the monkey cochlea.

MATERIAL AND METHODS

Six young, healthy Rhesus monkeys were used for this study. The cochlear vasculature was assessed following cochlear perfusion with contrast media. The technique has previously been described in detail (Axelsson, 1968, 1971, 1972). Briefly, the vascular system of the animal is perfused with Ringer's solution and subsequently with Prussian blue solution under static pressure. The temporal bones are fixed, decalcified, neutralized, washed, dehydrated, and stored in glycerin. The examination of the cochlea is

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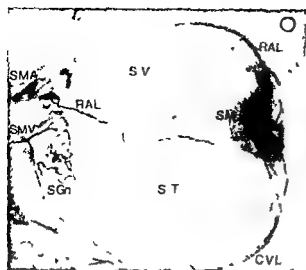


Fig 1 Basal turn of the Rhesus monkey cochlea, apico-basal section. The spiral modiolus artery (SMA) is situated at the level of the scala vestibuli (SV) and supplies the capillary areas in the external wall and the spiral lamina by radiating arterioles (RAL). The spiral modiolus vein (SMV) varies in course and is in this animal situated at the level of the spiral ganglion (SGn). SMV receives collecting venules (CVL) from all capillary areas. SM = scala media, ST = scala tympani.

done in the stereomicroscope and the photographic documentation made with the aid of the photomicroscope.

RESULTS

Modiolus

In the modiolus the following regularly occurring vessels can be identified in the Rhesus monkey:

- Spiral modiolus artery
- Spiral modiolus vein
- Radiating arterioles
- Collecting venules
- Capillaries in the spiral ganglion
- Capillaries in the acoustic nerve
- Capillaries in the modiolus wall

The Rhesus monkey cochlea has three turns. The spiral modiolus artery (SMA) derives from the common cochlear artery in the basal turn, about a fourth of a turn from the basal end of the cochlea. The common cochlear artery here divides into the vestibulo-cochlear artery and

the spiral modiolus artery. The spiral modiolus artery takes a serpentine course around the modiolus up to the cochlear apex and is situated, as in other mammals, at the level of the scala vestibuli of the modiolus (Fig 1). Numerous radiating arterioles (RAL) branch off from the spiral modiolus artery to supply the capillary areas of the external wall, the spiral lamina, as well as the spiral ganglion, VIII nerve, and the wall of the modiolus.

The spiral modiolus vein (SMV) is situated in the lower medial aspect of the scala tympani in the two apical turns. In the basal turn there appears to be variations in the course of the spiral modiolus vein. The vein sometimes appears in the lower medial aspect of the scala tympani but sometimes at the level of the scala vestibuli (Fig 1). In the latter case it receives large venous branches by a smaller vein of the lower medial aspect of the scala tympani. The collecting venules (CVL) derive from capillaries in the external wall, the spiral lamina, the spiral ganglion and the modiolus wall.

Similar to the other mammals, the VIIIth nerve (Fig 2) and the spiral ganglion (Fig 3) are supplied by many delicate capillaries without any regularly occurring arrangement. In the basal turn, the spiral modiolus vein has connections with delicate vessels in the bone surrounding the cochlea. The spiral modiolus vein empties in the region of the round window into the vein of the cochlear aqueduct. The vein of the



Fig 2 Capillaries of the acoustic nerve. Delicate capillaries run in different directions without any significant pattern.

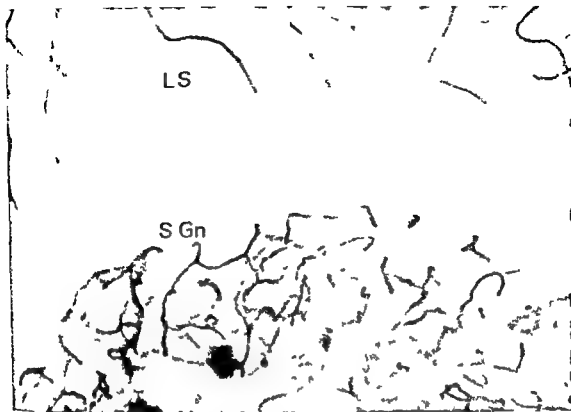


Fig 3 Spiral ganglion basal turn transverse section. Dense capillaries form a dense vascular net in the

spiral ganglion (SGn) without any specific arrangement. LS lamina spiralis.

cochlear aqueduct receives the vein of the round window and sometimes a separate vein of the scala tympani.

Spiral Lamina

In the Rhesus monkey the following vascular structures can be identified regularly

- Radiating arterioles
- Collecting venules
- The vessel of the tympanic lip
- The limbus vessels

Radiating arterioles (RAL) (Figs 1-4, 6) leave the spiral modiolar artery and have a coiled course centrally in the spiral lamina but straighten out more peripherally. They supply the capillary areas in the spiral limbus and the tympanic lip in a more or less T or Y formed manner. Each radiating arteriole supplies a fairly small segment of the peripheral vascular margins in

the tympanic lip and spiral limbus. The appearance of the radiating arterioles is very similar to that of the rabbit and human cochlea. The **collecting venules (CVL)** (Figs 1-4, 6) drain the spirally running capillary vessels at fairly right angles in a T or Y formed manner similarly to the radiating arterioles and run radially and centripetally over the spiral lamina to empty into the spiral modiolar vein. Often branches of the collecting venules anastomose centrally in the spiral lamina. The collecting venules also appear similar to those of the human rabbit and guinea pig cochlea. In all three turns the radiating arterioles and collecting venules also anastomose central to the vessel of the tympanic lip and form vascular arcades here (Figs 4, 6).

Infrequently small segments of the **vessel of the basilar membrane (VSBM)** (*vas spirale*) can be identified in the second and third turns of the monkey and very infrequently in the basal turn

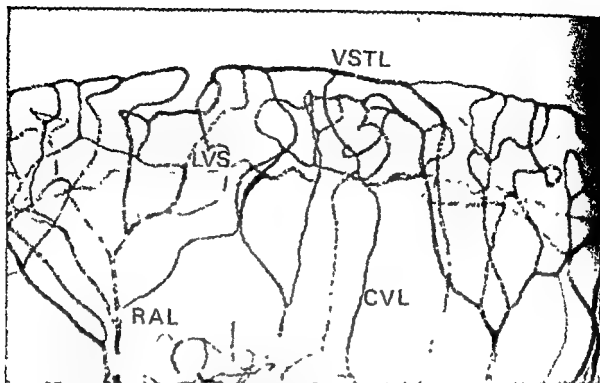


Fig 4 Spiral lamina, basal turn, transverse section. Vascular arcades form a spiral vascular margin the vessel of the tympanic lip (VSTL). There is no regularly occurring vessel under the outer hair cells. Radiating arterioles

(RAL) supply the capillary arcades (VSTL) and the limbus vessels (LVS). The vascular arcades are drained by collecting venules (CVL).

(Fig 5) This vessel does not occur regularly in the rabbit, but is found consistently in the guinea pig and human.

The vessel of the tympanic lip (VSTL) is situated at the level of the inner hair cells in the tympanic lip and constitutes the most peripheral spirally running capillary border of the spiral lamina (Figs 4, 5, 6). It is alternately supplied and drained by radiating arterioles and collecting venules. Often capillary arcades are seen centrally to the vessel of the tympanic lip particularly in the basal turn (Fig 4). These arcades may take a spiral course for short distances almost forming a spiral capillary border centrally and parallel to the vessel of the tympanic lip.

External Wall

Scala vestibuli

The following regularly occurring vessels could be demonstrated in the external wall.

Radiating arterioles

Collecting venules

Sparse capillary loops apical to the attachment of the vestibular membrane

The vessel at the vestibular membrane

The radiating arterioles (RAL) derive from the spiral modiolar artery and first run a serpentine centrifugal course over the scala vestibuli and then straighten more peripherally (Fig 8). The radiating arterioles supply all capillary areas in the external wall. The collecting venules (CVL) are more delicate and have a more irregular course than the radiating arterioles (Fig 8). They drain the capillaries and the vessel at the vestibular membrane. The collecting venules run both in an apical direction to the spiral modiolar vein of the next apical turn and in a basal direction to collecting venules from the scala tympani of the same turn externally to the stria vascularis. The vessel at the vestibular membrane (VSVM)

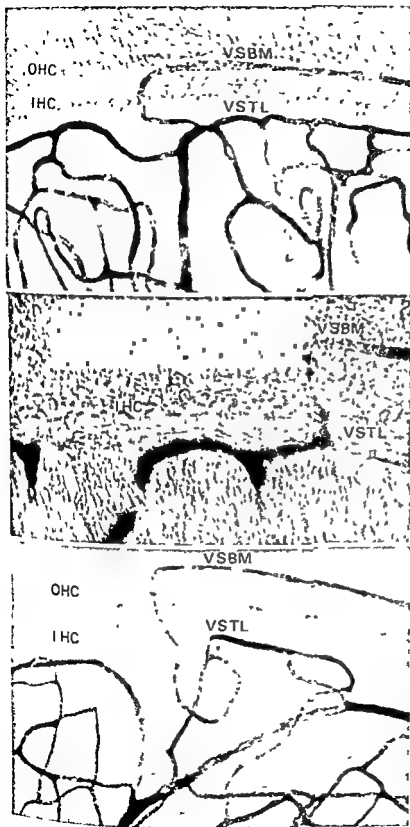


Fig 5 Spiral lamina, second turn, transverse section. An irregular vascular margin \equiv formed under the tunnel of Corti corresponding to the vessel of the basilar membrane (VSBM) VSTL = vessel of the tympanic lip OHC = outer hair cells IHC = inner hair cells.

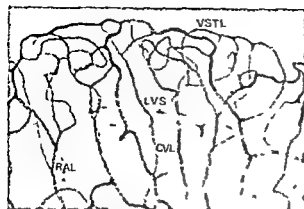


Fig 6 Spiral lamina second turn transverse section The peripheral vascular margin the vessel of the tympanic lip (VSTL), is less straight than in the basal turn There are many anastomoses between the radiating arterioles (RAL) and the collecting venules (CVL) central to the VSTL LVS = limbus vessels

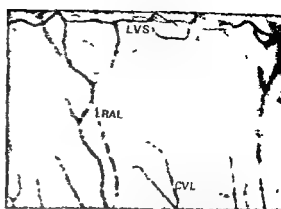


Fig 7 Spiral limbus, basal turn, transverse section Radiating arterioles (RAL) supply and collecting venule (CVL) drain the capillary vascular margin of the spiral limbus, the limbus vessels (LVS)

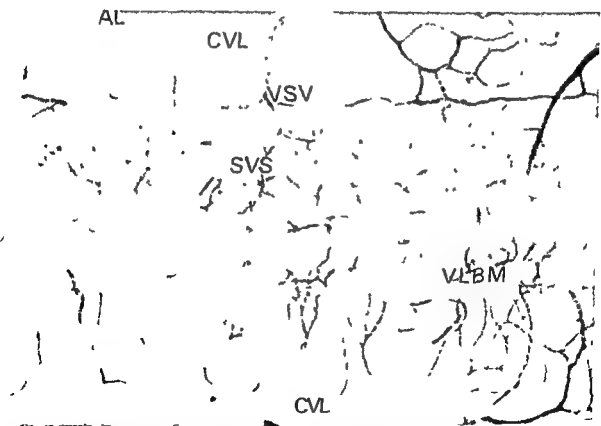


Fig 8 External wall basal turn apicobasal section Large radiating arterioles (RAL) supply and delicate collecting venules (CVL) drain the vessels in the scala vestibuli A basal vascular border is formed by the vessel at the vestibular membrane (VSV) The stria vascularis (SVS) and the vessel of the spiral prominence (VSP)

are equally supplied by the RAL The collecting venules (CVL) drain all vessels in the external wall and form a spirally running vessel immediately basal to the attachment of the basilar membrane the venules of the basilar membrane (VLBM)

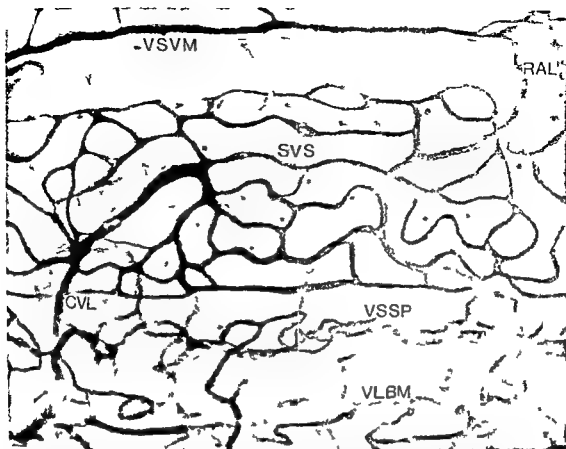


Fig 9 External wall basal turn ap cobasal section The vessel at the vestibular membrane (VSVM) is well formed. The stria vascularis (SVS) forms many capillary loops. The vessel of the spiral prominence (VSSP) is

made up of spirally running segments of capillaries. The venules at the basilar membrane (VLBM) are equally formed by spirally running portions of the collecting venules (CVL). RAL radiating arterioles.

is clearly evident as a spiral vascular border above the vestibular membrane in all three turns (Figs 8-9). The vessel at the vestibular membrane constitutes the basal border of the capillary loops in the scala vestibuli. The capillary loops of the scala vestibuli are formed by the sparse capillary connections of the finest branches from the radiating arterioles and collecting venules. They are too sparse to deserve the designation of a capillary net. There is no spirally running vessel of the scala vestibuli as it appears in the guinea pig.

Scala media

The following vascular structures can regularly be demonstrated in the scala media:

Stria vascularis

The vessel of the spiral prominence
Arteriovenous anastomoses

The stria vascularis (SVS) constitutes the most prominent vascular structure in the scala media containing a capillary net which includes a breadth of six to eight loops in the basal turn and one single loop in the third turn (Figs 8-9).

Stria vascularis is very similar to the same structure in the guinea pig rabbit and man. The stria vascularis is supplied by large but quite sparse radiating arterioles from the scala vestibuli and drained by equally large and sparse collecting venules derived from the scala tympani.

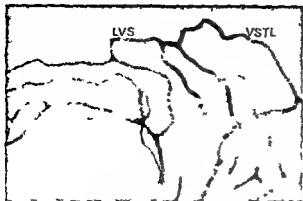


Fig 10 Spiral lamina apical end. The vascular pattern is much simplified and the vasculature terminates in simple vascular arcades. *VSTL* = vessel of the tympanic lip. *LVS* = limbus vessels. Helicotrema to the lower left.

(Fig. 8). There are no other vascular connections to the stria vascularis.

The vessel of the spiral prominence (*VSSP*) appears more irregular than in the guinea pig and is more similar to the corresponding vessel in man (Figs. 8, 9). It is formed from short segments of spirally running capillary vessels which are supplied by large and quite frequent radiating arterioles and drained by many collecting venules. There are no other vascular connections to the spiral prominence.

Arteriovenous anastomoses (*AVAS*) run external to the stria vascularis and the vessel of the spiral prominence. These vessels connect branches from the radiating arterioles in the scala vestibuli with collecting venules in the scala tympani (Figs. 8, 9). Similar to other mammals referred to above, the number of arteriovenous anastomoses is largest in the basal turn and least in the apical turn.

Scala tympani

The following vascular structures can regularly be demonstrated in the scala tympani:

Collecting venules

The venules at the basilar membrane

The collecting venules (*CVL*) are most numerous in the basal turn and decrease in frequency apically (Figs. 8, 9). They drain all the capillary areas in the external wall and anasto-

mose with radiating arterioles from the scala vestibuli externally to the stria vascularis. Similar to the other mammals investigated, the collecting venules seem to "grip" around the attachment of the basilar membrane in an omega formed manner. The collecting venules run a centripetal course over the scala tympani and often merge to form larger collecting venules basally in the scala tympani. The larger collecting venules empty into the spiral modiolar vein.

The venules at the basilar membrane (*VLBM*) are formed by the spirally running parts of the collecting venules immediately below the attachment of the basilar membrane in the external wall (Figs. 8, 9). In this way, a seemingly spirally running vessel is formed.

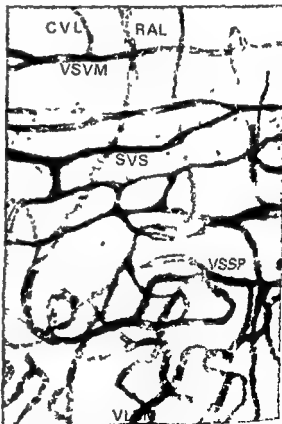


Fig. 11 External wall second turn, apicobasal section. The stria vascularis (*SVS*) shows an increasing narrowing towards the apex. The vascular pattern is otherwise maintained. *RAL* = radiating arteriole. *CVL* = collecting venule. *VSVM* = vessel at the vestibular membrane. *VSSP* = vessel of the spiral prominence. *VLBM* = venules at the basilar membrane.

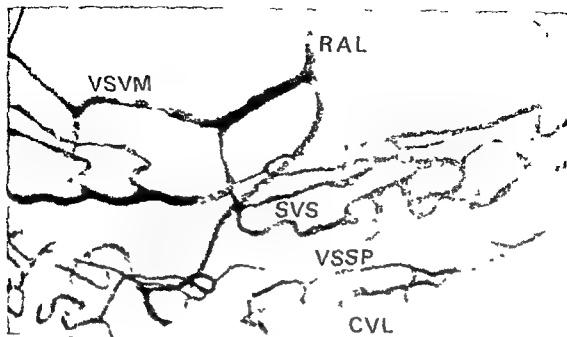


Fig 13 External wall apical end apicobasal section. The vascular pattern is much simplified. Many of the regularly occurring vessels can still be identified. RAL =

radiating arteriole. VSVM = vessel at the vestibular membrane. SVS = stria vascularis. VSSP = vessel of the spiral prominence. CVL = collecting venules.

Apical end

Similar to man, rabbit, and guinea pig, the apical end is characterized by a seemingly pronounced sparseness of the vasculature. In the spiral lamina (Fig 10) the vessel of the tympanic lip can be identified up to the apical end, and there is often a vessel of the basilar membrane present in the most apical part. The limbus vessels run a more wavy course than more basally. In the external wall (Figs 11, 12) the number of arteriovenous anastomoses decrease. The radiating arterioles particularly supply the stria vascularis and the vessel of the spiral prominence which are the most prominent capillary structures at the level of the apex. The vessel at the vestibular membrane runs straight spirally and is well maintained up to the apical end.

Basal end

In the basal end, the radiating arterioles (RAL) deriving from the vestibulo-cochlear artery run an oblique course towards the scala vestibuli in the region of the oval window (Fig 13). When

the vestibulo-cochlear artery arrives in the vestibulum one or two arterial branches (RAL) run around the extreme basal end of the spiral lamina and return 'upward' to merge with radiating arterioles also running obliquely over the scala vestibuli from the apical cochlear side of the oval window. A capillary net is formed in the scala vestibuli where the branches of the two radiating arterioles merge from opposite directions. In the scala tympani the collecting venules (CVL) drain into the vein of the round window which runs around the apical aspect of the round window, similar to the other mammals investigated. The capillary vessels in the spiral lamina and the external wall are well formed to the extreme basal end (Fig 13).

DISCUSSION

The Rhesus monkey has recently become a frequently used mammal for cochlear research. Consequently, basic anatomical information concerning all parts of the cochlea is needed.

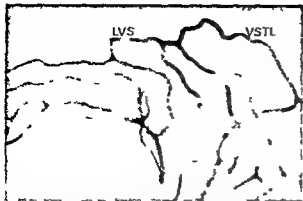


Fig 10 Spiral lamina, apical end. The vascular pattern is much simplified and the vasculature terminates in simple vascular arcades. VSTL = vessel of the tympanic lip. LVS = limbus vessels. Helicotrema to the lower left.

(Fig 8). There are no other vascular connections to the stria vascularis.

The vessel of the spiral prominence (VSSP) appears more irregular than in the guinea pig and is more similar to the corresponding vessel in man (Figs 8, 9). It is formed from short segments of spirally running capillary vessels which are supplied by large and quite frequent radiating arterioles and drained by many collecting venules. There are no other vascular connections to the spiral prominence.

Arteriovenous anastomoses (AVAS) run external to the stria vascularis and the vessel of the spiral prominence. These vessels connect branches from the radiating arterioles in the scala vestibuli with collecting venules in the scala tympani (Figs 8, 9). Similar to other mammals referred to above, the number of arteriovenous anastomoses is largest in the basal turn and least in the apical turn.

Scala tympani

The following vascular structures can regularly be demonstrated in the scala tympani:

Collecting venules

The venules at the basilar membrane

The collecting venules (CVL) are most numerous in the basal turn and decrease in frequency apically (Figs 8, 9). They drain all the capillary areas in the external wall and anasto-

mose with radiating arterioles from the scala vestibuli externally to the stria vascularis. Similar to the other mammals investigated, the collecting venules seem to "grip" around the attachment of the basilar membrane in an omega formed manner. The collecting venules run a centripetal course over the scala tympani and often merge to form larger collecting venules basally in the scala tympani. The larger collecting venules empty into the spiral modiolar vein.

The venules at the basilar membrane (VLBM) are formed by the spirally running parts of the collecting venules immediately below the attachment of the basilar membrane in the external wall (Figs 8, 9). In this way, a seemingly spirally running vessel is formed.

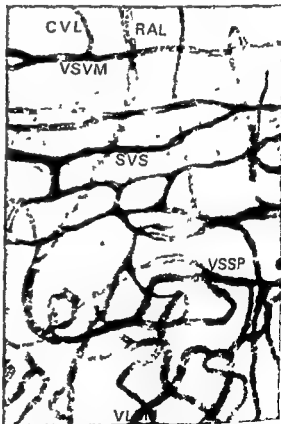


Fig 11 External wall, second turn, apicobasal section. The stria vascularis (SVS) shows an increasing narrowing towards the apex. The vascular pattern is otherwise maintained. RAL = radiating arteriole. CVL = collecting venule. VSSP = vessel at the vestibular membrane. VLBM = venules at the basilar membrane.

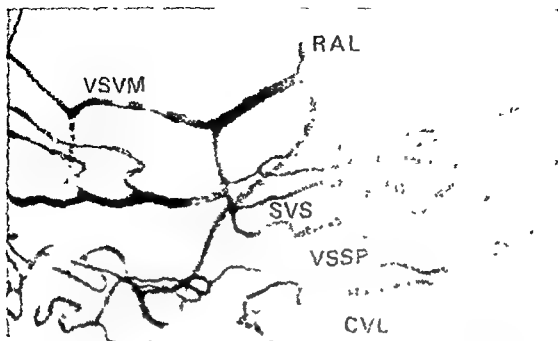


Fig. 12. External wall, apical end, apicobasal section. The vascular pattern is much simplified. Many of the regularly occurring vessels can still be identified: RAL, radiating arteriole; VSVM, vessel at the vestibular membrane; SVS, stria vascularis; VSSP, vessel of the spiral prominence; CVL, collecting venules.

radiating arteriole; VSVM = vessel at the vestibular membrane; SVS = stria vascularis; VSSP = vessel of the spiral prominence; CVL = collecting venules.

Apical end

Similar to man, rabbit, and guinea pig, the apical end is characterized by a seemingly pronounced sparseness of the vasculature. In the spiral lamina (Fig. 10) the vessel of the tympanic lip can be identified up to the apical end, and there is often a vessel of the basilar membrane present in the most apical part. The limbus vessels run a more uneven course than more basally. In the external wall (Figs 11, 12), the number of arteriovenous anastomoses decrease. The radiating arterioles particularly supply the stria vascularis and the vessel of the spiral prominence which are the most prominent capillary structures at the level of the apex. The vessel at the vestibular membrane runs straight spirally and is well maintained up to the apical end.

Basal end

In the basal end, the radiating arterioles (RAL) deriving from the vestibulo-cochlear artery run an oblique course towards the scala vestibuli in the region of the oval window (Fig. 13). When

the vestibulo-cochlear artery arrives in the vestibulum one or two arterial branches (RAL) run around the extreme basal end of the spiral lamina and return 'upward' to merge with radiating arterioles, also running obliquely over the scala vestibuli from the apical, cochlear side of the oval window. A capillary net is formed in the scala vestibuli where the branches of the two radiating arterioles merge from opposite directions. In the scala tympani the collecting venules (CVL) drain into the vein of the round window which runs around the apical aspect of the round window, similar to the other mammals investigated. The capillary vessels in the spiral lamina and the external wall are well-formed to the extreme basal end (Fig. 13).

DISCUSSION

The Rhesus monkey has recently become a frequently used mammal for cochlear research. Consequently, basic anatomical information concerning all parts of the cochlea is needed.

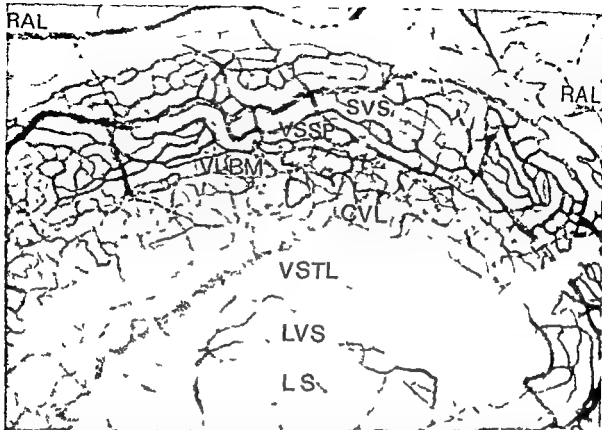


Fig 13 Basal end. In the region between the oval window and the round window the radiating arterioles (RAL) coming from the cochlear side (left) merge with radiating arterioles coming from the vestibulum (right). The vascular tributaries of the external wall are well formed here.

SVS = stria vascularis, VSSP = vessel of the spiral prominence, VLBM = venules at the basilar membrane, CVL = collecting venules. In the lower part the sparse vasculature of the spiral lamina (LS) can be seen. VSTL = vessel of the tympanic lip, LVS = limbus vessels.

From the technical viewpoint, the Rhesus monkey is easily injected and yields consistent good results at least with the present small numbers (six animals, twelve cochleas). The removal of the temporal bones after injection requires more time than in either the guinea pig or the rabbit and amounts to three to six minutes. The subsequent technical steps were easily achieved and all parts of the cochlea can be demonstrated in surface specimen preparation, just as easily as in the guinea pig or rabbit cochlea.

The nomenclature adopted on the cochlear vessels is based on the relation of vascular structures to other anatomical structures or on the course of the vessels in the cochlea. The nomenclature consequently is not based on histological examinations of the vascular wall which may raise disagreements in naming vessels. An arteri-

ole, i.e. is here looked upon as a vessel connecting a large supplying artery with the terminal vascular bed where the vessels are termed capillaries regardless of their morphological structure. Equally, a collecting venule is considered as a vascular structure connecting the capillaries with a vein etc.

In general the vascular anatomy of the Rhesus monkey cochlea is very similar to the human guinea pig and rabbit (Axelsson 1968, 1971; Axelsson & Lind, 1973). The main characteristics of the vascular anatomy in the Rhesus monkey are:

The arterial system including larger arteries as well as radiating arterioles is similar to the other mammalian species investigated so far (man, rabbit, guinea pig).

The venous system is more irregular than in the guinea pig and rabbit and is more similar to the human cochlea in showing variations particularly in the basal turn.

The collecting venules in the external wall merge to large collecting veins more peripheral in the scala tympani than in the human, rabbit or guinea pig cochlea.

The vessel of the basilar membrane only occurs irregularly and most often in the third and second turn. In this respect the Rhesus monkey cochlea is similar to the rabbit cochlea. The spirally running capillary vascular border in the tympanic lip and in the spiral limbus is similar to the other mammals investigated.

In the external wall there is only a very sparse capillary net in the scala vestibuli and no spirally running vessel of the scala vestibuli as in the guinea pig. The capillary areas in the external wall otherwise are similar to the human, rabbit and guinea pig cochlea.

At the basal end (the hook region) all vascular tributaries in the external wall are well formed and more so than in the human, rabbit and guinea pig cochleas.

As reviewed in the introduction, very sparse information is available concerning the vasculature of the monkey cochlea. The main point of controversy seems to be the number of spiral vessels in the spiral lamina. In the present investigation, the principal finding was one spiral capillary vessel of the tympanic lip and an infrequent occurrence of the vessel of the basilar membrane. When this vessel could be demonstrated it was most frequently found in the second and third turn. As stated from several previous investigations the vessel of the basilar membrane might be of its greatest importance during fetal life with a subsequent involution (Hawkins & Johnsson, 1968; Hilding 1969; Johnsson, 1972; Johnsson & Hawkins 1972). This also suggests that the importance of this vessel for the oxygen supply to the organ of Corti may be less pronounced in postfetal life than suggested by many authors. This is also suggested by the finding that several species of mammals lack

this vessel, i.e. rabbit (Retzius, 1884; Nabeya, 1923; Axelsson & Lind, 1973) cat (Smith 1954) and mink (Hilding et al., 1967).

The venous system of the modiolus appears more irregular than in the guinea pig and rabbit and consequently is more similar to the complicated and largely individually varied appearance of the human cochlea. The sparse connections between the veins of the modiolus and the surrounding bone vessels are not of the size or frequency to indicate any function of significant importance. The favorable outcome of the injections and the inconsistency of a capillary vessel under the outer hair cells make the Rhesus monkey a most interesting experimental animal for cochlear research.

ACKNOWLEDGMENT

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ZUSAMMENFASSUNG

Das Gefässsystem der Rhesusaffen Cochlea wurde mit Hilfe von Kontrastmittelinjektion dargestellt. Die Kapillarnetze sind denen des Menschen, des Kaninchens und Meerschweinchens sehr ähnlich. Ein Hauptunterschied ist ein unregelmässiges Vorhandensein des Gefässes unter dem Cortischen Tunnel. Die Kapillarnetze der Aussenwand am basalen Cochlea Ende haben eine grössere Gefässversorgung als beim Menschen, Kaninchen und Meerschweinchen. Der Verlauf und die Verzweigung der Venen im Modiolus sind unregelmässig und ähneln der menschlichen Cochlea mehr als der des Kaninchens und Meerschweinchens. Das unregelmässige Vorhandensein des Gefässes unter dem Cortischen Tunnel und der ausseren Haarzellen macht die Rhesusaffen Cochlea besonders interessant für die Gehörforschung.

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DISTRIBUTION OF THE CROSSED OLIVO-COCHLEAR BUNDLE TERMINALS IN THE SQUIRREL MONKEY COCHLEA

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Abstract Distribution of the crossed olivo-cochlear bundle terminals in the squirrel monkey cochlea was studied electron microscopically after transection of the bundle on the floor of the fourth ventricle. About 70% of the inner spiral bundle belongs to the crossed olivo-cochlear bundle at the lower basal turns. About 90% of all efferent endings to the outer hair cells belong to the crossed olivo-cochlear bundle within the lower basal turns. The innervation percentage of the crossed olivo-cochlear bundle is relatively reduced within the upper turns. After studying the serial transverse sections of the brain stem, it was found that all of the midline sections extended well beyond the edge of the facial genu both rostrally and caudally.

The olivo-cochlear bundle (OCB), which is a part of the auditory efferent system from the brain stem to the organ of Corti, was first described by Rasmussen in the cat. Since that time, the course of this bundle and distribution patterns of peripheral terminals within the organ of Corti have been studied in many different animal species. The histochemical approach using acetylcholinesterase staining, etc., the electron microscopic investigation of peripheral terminals, and the nerve degeneration studies by Nauta staining were commonly used (Rasmussen, 1953, Schuknecht et al., 1959, Hilding & Wersäll, 1962, Gacek et al., 1965, Terayama & Yamamoto, 1971, Spoendlin, 1967). The distri-

bution patterns of the OCB peripheral terminals within the organ of Corti were electron microscopically studied by Kimura & Wersäll (1962) in the guinea pig, by Iurato (1962) in the rat, by Smith & Rasmussen (1963, 1965) and Iurato et al. (1968) in the chinchilla, and by Spoendlin & Gacek (1963) in the cat.

By utilizing avoidance behavioral conditioning, the authors have studied the psychophysiological contribution of the crossed OCB system to various aspects of systemic auditory function (Igarashi et al., 1972). However, as the OCB peripheral distribution pattern varies slightly among different animal species, and as the psychobiological importance of this OCB system should differ from species to species, there is reason to extend the investigation to the experimental primate.

The main purpose of the present investigation is to set up the first step in utilizing primate subjects for investigation. In the first step of this study, the distribution pattern of the crossed OCB terminals within the organ of Corti was electron microscopically investigated by utilizing normal adult squirrel monkeys.

METHODS

Ten young adult squirrel monkeys, weighing 500-700 grams, free from any middle ear disorders (based upon otoscopic observation and confirmation at the time of removal of electron

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microscopic specimens) were used as the experimental animals in the present investigation.

The intraperitoneal injection of sodium pentobarbital (30 mg/kg) was used for the animal anesthesia. The occipital midline incision was made and the bony calvarium opened to expose the posterior portion of the cerebellum and brain stem. The cerebellum (mostly the area of the uvula) was gently elevated by a non rigid metal retractor. By using a fine pick, the crossed OCB was sectioned at the region of the facial genu in the floor of the fourth ventricle. The midline incision was intentionally extended rostrally and caudally at least 1–2 mm beyond the edge of the facial genu. During this procedure, care was taken not to retract the cerebellum too much or for too long. All bleeding spots were controlled, the area covered by a thin piece of gelfoam, and the wound closed.

The animals were sacrificed 14 days after surgery, except for one subject after 7 days, one after 17 days and two after 24 days. The monkeys were deeply anesthetized and the inner ear was approached retroauricularly. The perilymphatic scalae were perfused with 2% osmium tetroxide solution. After decapitation, the temporal bones were removed and further immersed in the identical fixative and refrigerated for 2 hours. Then the specimens were dehydrated in graded alcohol and embedded in Epon plastic. A fine saw was used to divide each turn into several different blocks. These were mounted on Epon blanks for sectioning. Free hand sections were made for light microscopic study, a Porter-Blum Ultratome was used for making thin sections. Ultra thin sections were stained with uranyl acetate and lead citrate, and the study was done under a JEM-7 electron microscope.

The serial transverse sections (20 μ m) of the brain stem were made by cryostat, and Nauta staining was performed in order to investigate the nerve degeneration and also the depth and extent of the surgical lesion.

RESULTS

Severe nerve fiber degeneration within the organ of Corti was observed in the subjects two weeks

after the transection of crossed OCB. In the nerve endings to the outer hair cells, accumulation or reduction of vesicles, vacuole formation, swelling of mitochondria, rupture of the membrane and appearance of osmophilic substance, were observed to variable extents and degrees (Figs 1, 2, 2A, 2B, and 2C). These degeneration findings resemble those previously reported in the guinea pig and cat. Three and one half weeks after the OCB transection, the complete disappearance of the nerve ending was observed in some areas. Even though the nerve ending disappeared completely, the subsynaptic cisterna remained in the outer hair cells. Degenerated cell debris (which was floating within the cortical lymph space) was also found.

Fiber reduction of the inner spiral bundle was found in the basal turn. Thus, the existence of OCB in that particular region was confirmed (Fig 3). Likewise, few crossed OCB nerve endings were found at the inferior portion of the inner hair cells (Fig 4).

Degeneration of the tunnel spiral bundle was clearly observed (Fig 6). More degeneration was found in the lower turns than in the upper turns.

With regard to the radial fibers within the Corti tunnel, interspecies difference exists. In the guinea pig, for example, both efferent and afferent nerve fibers exist together. In the cat, the tunnel radial fibers are considered to be basically efferent fibers, the afferent nerve fibers run inferiorly along the basal membrane, being surrounded by pillar cells. The present findings in the squirrel monkey resemble those in the cat. The upper radial fibers belong to the efferent fibers (Fig 7) and a part of the afferent fibers run inferiorly, being surrounded by pillar cells (Fig 8).

The majority of the efferent nerve endings (which are attached to the inferior portion of the outer hair cells) in the lower basal turn belong to the crossed OCB. Within the upper basal turn, about a half of the nerve endings belong to the crossed OCB, however, crossed OCB terminals are scarce at the middle turn and above.

Within the upper basal to middle turns (espe-



Fig 1 Electronmicrograph exhibits the different forms of nerve endings on an outer hair cell (H). Two large nerve endings (E) which contain vesicles and mitochondria,

and one small nerve ending (A) can be seen. Squirrel monkey normal subject. The black scale bar indicates 1 μm (in all figures).

cially 15–20 mm from the basal end) some of different nerve endings attach to the supranuclear portion of the outer hair cells. These particular nerve endings belong to the crossed OCB. This finding is considered to be specific to the squirrel monkey (Fig 9).

In summarizing the above described findings, about 70% of the inner spiral bundle in the lower basal turn belong to the crossed OCB. Approximately the same percentage was found in the tunnel spiral bundle. Some nerve endings

of the crossed OCB exist around the inner hair cells. In general, within the upper turns there is a reduction of the crossed OCB endings.

Regarding the nerve terminals attached to the outer hair cells, about 90% belong to the crossed OCB at the lower basal turns, about 50% or more at the upper basal turns, however, at the middle turn and above most of the nerve endings belong to homolateral OCB (Fig 10).

After studying the serial transverse sections of the brain stems of squirrel monkeys, it was

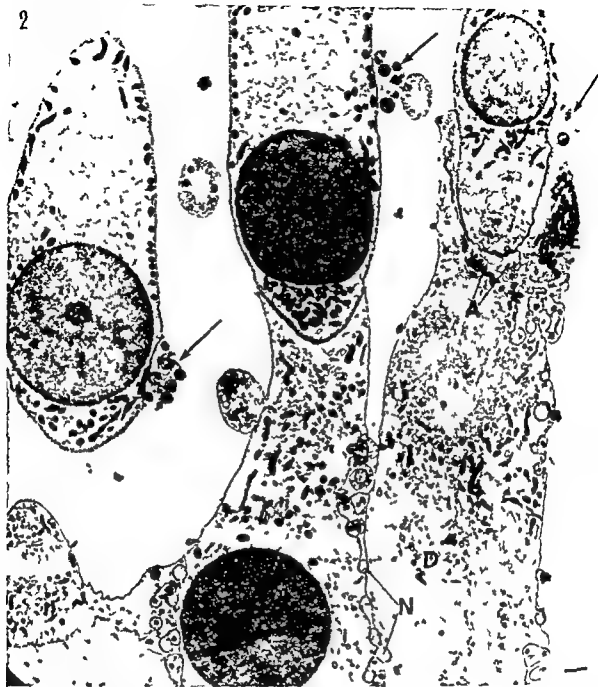


Fig. 2 This electron micrograph demonstrates that three nerve endings (arrows) are in an advanced stage of degeneration 2 weeks after the transection of the crossed OCB. This figure displays the area of outer hair cells from the upper basal turn (about 10 mm from the basal

end). The one efferent (probably ipsilateral) nerve ending (E) and afferent nerve endings (A) at the right have an intact appearance. N: afferent nerve fibers; D: debris cell. Squirel monkey B9E.



Fig. 2A-C Higher magnification views of parts of Fig. 2 showing the degenerate trans-lateral efferent nerve endings on the outer hair cells.



found that the facial genu were relatively small in size and were usually located between 5 and 7 mm from the obex (Fig. 11). It was found that all of the midline sections were extended well beyond the level of the edge of the facial genu (1–2 mm), both rostrally and caudally. Therefore, if the crossed OCB goes across the midline at the region of the facial genu, it can be assumed that the majority of the crossed OCB were properly cut. Out of 10 subjects, degenerated nerves (immediately beneath the facial genu) were seen in 3 and another 3 showed uncertain but possible degeneration.

DISCUSSION

Studies in other animal species have shown that the large nerve endings of the outer hair cells degenerate after cutting the OCB. Kimura & Wersall (1962) found degeneration in some of the large nerve endings as well as some afferents to outer hair cells and some nerves in the tunnel bundle in the guinea pig after transection of crossed OCB. Iurato (1962) reported that the large nerve endings disappeared within 8 days after the OCB section in the rat. Smith & Rasmussen (1963, 1965) by using the chinchilla, studied the degeneration behavior of both translateral and ipsilateral OCB between 2 and 35 days post transection. They reported that the initial degeneration sign could be found within nerve fibers and large nerve endings 3 to 4 days post transection, and almost complete disappearance of cell debris 21 days post operation. Spoendlin & Gacek (1963) also studied the efferent nerve system in the cat cochlea after cutting the homo- and contralateral OCB. Spoendlin (1969) further found that not only had a great number of the efferent endings at the outer hair cells degenerated but also the number of internal spiral fibers seemed to be reduced after the transection of the crossed OCB. Inasmuch as inter-species

difference exists, there is reason to utilize higher mammals such as subhuman primates for the study of OCB function.

In the cat, approximately 2 weeks after the transection, the terminal degeneration was usually complete, with the exception of occasional small cell debris. The nerve endings and fibers had completely disappeared (Spoendlin 1969; Igarashi et al., 1972). In the present study we have investigated the squirrel monkey cochlea 1–3½ weeks after the transection of the contralateral OCB. The obtained results were fairly similar to those of the cat studies and the terminal degeneration was usually complete 2 weeks after the transection. In the two cases 3½ weeks after the transection, nerve endings and fibers had almost completely disappeared; therefore, it was considered that the survival time period in the present study was the appropriate time to investigate the distribution pattern of the OCB terminals in the squirrel monkey.

Iurato (1962) described how the crossed OCB innervates only the outer hair cells in the rat. According to Rasmussen's report (1960) approximately 4/5 of the fiber population of the OCB originate in the crossed bundle from the contralateral accessory olivary nucleus, the remaining 1/5 originate at the uncrossed bundle from the homolateral 8 shaped olivary segment in the cat.

In the present investigation of the squirrel monkey, it was found that about 70% of the inner spiral bundle belongs to the crossed OCB at the lower basal turns. The crossed OCB partly innervates inner hair cells (directly). About 90% of all efferent endings to the outer hair cells belong to the crossed OCB within the lower basal turns. The innervation percentage of the crossed OCB relatively decreased within the upper turns, and the population from the homolateral OCB increased.

Fig. 3 Electronmicrograph demonstrates the lower portion of the inner hair cell. The specimen was obtained about 6 mm from the basal end. Inner spiral bundles below the inner hair cell (IH) have partly disappeared 2 weeks post OCB transection. Squirrel monkey D9A.

Fig. 4 This electronmicrograph exhibits the lower portion of the inner hair cell (IH). The specimen was obtained about 10 mm from the basal end 2 weeks post OCB transection. Squirrel monkey A9B. A nerve ending (E) has disintegrated P pillar cell.

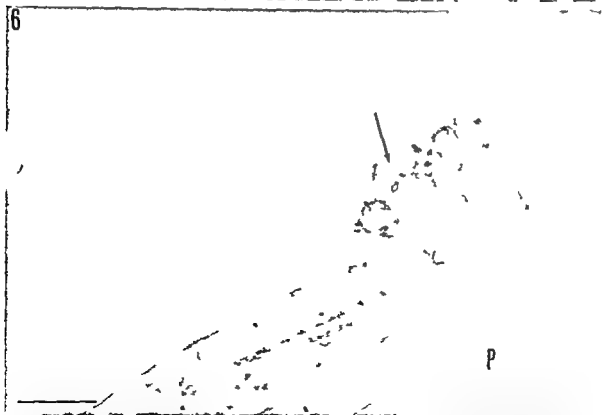


Fig 5 Electron micrograph demonstrates tunnel spiral bundle (arrow) P pillar cell Squirel monkey normal subject Fig 6 The electron micrograph exhibits the condition of the tunnel spiral bundle 2 weeks after the OCB transect on Not a degenerating efferent nerve fibers which belong to the crossed OCB (arrow) The specimen was obtained from an area about 8 mm from the basal end. P pillar cell Squirel monkey A9B



Fig 7 This electron micrograph demonstrates the degenerative changes in the part of the tunnel radial fibers (arrow). The specimen was obtained about 15 mm from the basal end some 3½ weeks post OCB transect on Squirrel monkey B3B

Fig 8 Electron micrograph shows the tunnel basal fiber (arrow) which is entirely embedded in invagination of the pillar cells (P) Squirrel monkey B2B



Fig 9 Electronmicrograph demonstrates a disintegrated nerve ending (arrow) in the supranuclear portion of an outer hair cell (H). The subsynaptic cisterna along the hair cell membrane appears to be normal. Other efferent (E) and afferent (A) endings show no change. About 20 mm from the basal end, 2 weeks post OCB transection. Squirrel monkey B2E. (A) Higher magnification view of the disintegrated nerve ending (arrow) in Fig 9.

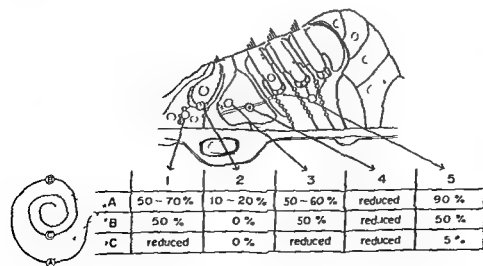


Fig 10 Innervation from crossed olivo-cochlear bundle

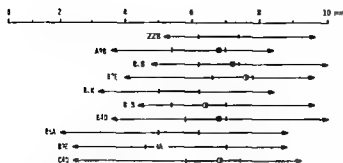


Fig 11 This figure displays the rostro-caudal extent of midline lesions in squirrel monkeys. The abscissa indicates the rostral distance from the obex. Heavy line segments indicate the area of facial genu nerve degeneration (by Nauta staining) was seen in cases with black circles while half black circles indicate possible degeneration (A) artefact

The degenerating condition or disappearing percentage of nerve fibers and endings within the organ of Corti varied among the different individuals. One reason for this is probably the different extent of the midline sections at the brain stem or this is certainly due to the intra-individual variance.

After the OCB transection in the cat, Spoendlin & Gacek (1963) and Spoendlin (1969) concluded that the efferent exist only in the upper tunnel radial bundle while the basilar bundle belongs to the afferent. At the floor of the Corti tunnel, the basilar afferent fibers are usually embedded in invagination of the pillar cells, most of these take a short spiral course before they pass between the outer pillars. In the guinea pig there are afferent fibers crossing the tunnel at a higher level according to Kimura & Wersäll (1962).

In the present study of the squirrel monkey, some of the basilar fibers are also embedded in invagination of the pillar cells just as in the cat, therefore, the basilar fibers might be afferent in nature. On the other hand, some of the tunnel radial fibers degenerate after the midline transection, therefore, these radial fibers are considered to be efferent in nature. Ishii et al (1967) described that both the upper tunnel radial bundle and the basilar radial bundle seemed to contain intense acetylcholinesterase activity in the squirrel monkey, therefore they suspected that both of these tunnel bundles contained efferent fibers in the squirrel monkey. The discrepancy of this point remains to be determined.

The nerve endings to the outer hair cells

usually attach to the inferior portion of the cell body. However, in the present study of the squirrel monkey, some of the efferent nerve endings attached to the supra-nuclear region of the outer hair cells at the upper basal and middle turns. All of these endings belonged to the crossed OCB. No such description was found in the rat, mouse, guinea pig, chinchilla or cat, therefore, it is considered that these supra nuclear nerve endings might be specific to the squirrel monkey.

ZUSAMMENFASSUNG

Bei einem Totenkopffaffen (*Saimiri sciureus*) wurde nach einem Querschnitt des Bündels am Boden der vierten Kammer die Verteilung der Enden des gekreuzten Olivo-Cochlea-Bündels untersucht, und zwar elektronenmikroskopisch. Ungefähr 70% des inneren Spiralen-Bündels gehörten zu dem gekreuzten Olivo-Cochlea-Bündel der unteren Grundspiralen. Ungefähr 90% aller Enden, die nach aussen zu den äusseren Haarzellen führen, gehören zu dem gekreuzten Olivo-Cochlea-Bündel innerhalb der unteren Grundspiralen. Der Prozentsatz der Nervenverteilung des gekreuzten Olivo-Cochlea-Bündels ist dementsprechend geringes innerhalb der oberen Spiralen. Nach Untersuchung von 100000 Gesichtsfeldern des Cochleamagmas.

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SOURCE OF THE P_1 COMPONENT OF THE COCHLEA ROUND WINDOW RECORDING

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Abstract The cochlear round window potentials of cats and guinea pigs have been studied in their response to a short click stimulus coupled with specifically placed neural lesions. With the destruction of a specific area of

observation in contrast to the extensive recovery observed within 2 hours after lesions were made at the internal auditory meatus (including the vestibular nerve and OCB fibers). The lack of recovery in the animals in which lesions were placed in the cochlear nucleus may be explained on the basis that the responsible neurons in this area were destroyed. Since the P_1 potential has a short latency with respect to the termination of microphonic activity and the changes are seen only with lesions placed peripherally to or at the specific cochlear nucleus area it is proposed that the P_1 potential possibly represents an activity of a peripheral coding or inhibitory mechanism.

Davis 1957 presented an interpretation of the recordings obtained from the scalae of the cochlea and from the round window. Many investigations of the cochlea have been based upon the use of these various electrical potentials recorded from the cochlea. As the application of these methods is extended it has become evident that limitations exist concerning their interpretation. During the past few years a number of experiments have been published dealing with the extension of the knowledge of what these recordings may represent (Dallos et al, 1971; Lawrence 1959; Salomon & Elberling 1971; Sohmer et al, 1971).

From this study evidence is offered towards

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extending the information concerning the usefulness of volume recording from the round window (rw) in response to a short click stimulus. It is recognized that volume recordings are an averaging of all the active components in the recording area under the electrode. Consequently the recording has limitation when the components are to be interpreted. Although this is the case with the potentials we are dealing with it is proposed that when a particular portion of the tracing is consistently affected by an experimental change it is possible to assign some specific correlate to it. Our interest in these experiments is particularly concerned with the positive (P_1) component. We have studied the influence of placing lesions along different parts of the efferent innervation at the level of the midbrain. It is the aim of this study to learn whether the P_1 potential is associated with any of these sites.

The interest of the ENT Department clinical personnel in the use of human intra aural cochlear potential recordings as a diagnostic aid has been fostered due to the development of signal averaging techniques (cochleograms). Therefore it was deemed important that what information we obtained concerning the possible interpretation of these potentials should be presented.

METHODS

Animal lesion surgery

The experimental animals, cats (20) and guinea pigs (9) were anesthetized with intraperitoneal

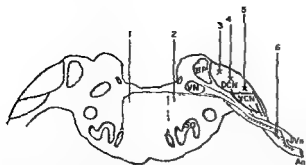


Fig 1 Diagrammatic representation of the placement of various lesions in the cat auditory innervation. Lesion 1 is placed to interrupt the contralateral olivocochlear fiber, lesion 2 placement will section both ipsilateral and contralateral olivocochlear bundle fibers, lesion 3 is in the medial pole of the cochlear nucleus as observed from a dorsal exposure, lesion 4 is placed in the cochlear nucleus mid portion of the dorsal exposure, lesion 5 is placed at the lateral pole of the observable field and lesion 6 is a section of the vestibular nerve at the internal meatus

injections of 0.7 ml/kg Dial Urethane solution (Ciba). They were placed into a Kopf stereotaxic head holder for orientation purposes. A posterior fossa craniotomy was performed, by carefully removing occipital bone from the occipital crest to the foramen magnum and laterally out to the occipital condylus. After splitting the dura, over the cerebellum, the cerebellum was removed by suction to expose the fourth ventricle and adjoining parts.

This exposure allowed visualization of the dorsal surface of the VIII nerve from its emergence rostral-ventral to the cochlear nucleus.

Lesions were placed as shown in Fig 1. The sequence of lesions (refer to Fig 2) in each group of animals was performed as follows: lesion 6 alone with a wire loop (5 cats), lesions 1, 2, 3, 4 and then 5 performed with a wire loop (6 cats, 5 guinea pigs), lesions 3, 4 and 5 performed with a bipolar (1 mm distance) electrode, current 2 milliamps, duration 15 sec (5 cats), lesions 3, 4 and then 5 performed with the injection of a phenol solution (0.02 ml of 20% phenol in glycerin) with a 27 gauge hypodermic needle (4 cats) and the sectioning of the VIII nerve at the internal meatus with a wire loop (4 guinea pigs).

Recording procedure

The round window potential measurement was obtained with a unipolar silver tipped spring loaded electrode placed at the base of the round window. The approach was made through a lateral exposure of the bulla while the indifferent electrode was attached to the cranium. The animal was grounded through the mouth clamp. The cochlea round window potential was amplified with a Grass P9 capacity coupled preamplifier and recorded on a Tektronix Model 565 Oscilloscope. Using the control tracing as a guide the time relationships for N_1 and P_1 peak voltage were determined (Fig 3, 1, 2 and 16 msec respectively from the onset of the microphonics). These times were then used to locate the approx

The μV Value of Cochlear N_1 and P_1 Potentials Following Lesions Placed in Various Parts of the Cochlear Innervation








Methods of Lesions		Placements						
		Control	1 COCB	2 COCB HOCB	3 Coch Nuc med of	4 Coch Nuc lat of	5 Coch Nuc Lateral	6 Vest Nerve
Wire Section	N	357 ± 82	156 ± 30	164 ± 43	164 ± 37	148 ± 40	220 ± 80	436 ± 106
	P	184 ± 50	70 ± 23	78 ± 34	70 ± 30	68 ± 25	96 ± 20	11 ± 33
Electrical Coagulation	N	190 ± 30			235 ± 73	210 ± 50	285 ± 66	
	P	43 ± 2			202 ± 56	5 ± 37	120 ± 65	
Phenol Injection	N	276 ± 33			300 ± 47	332 ± 39	362 ± 70	
	P	123 ± 17			120 ± 12	129 ± 15	97 ± 10	
Representative Tracing								

Fig 2 These are the mean values \pm standard error of the amplitude (μV) of the negative potential (N_1) and the positive potential (P_1) recorded from the cat cochlea round window. Each group of animals served as their own controls. The lesion placements are as indicated in Fig 1. The tracing inserts are examples of the potentials obtained from the cochlea under the condition indicated. The N_1 amplitude and delay were not significantly changed by the lesions which produced P_1 alterations. The abbreviations used are COCB (contralateral olivocochlear bundle), HOCB (homolateral olivocochlear bundle), Coch nuc (cochlear nucleus), vest. (vestibular), \square (wire loop lesions experiment series), \square (electrical coagulation experiment series).

ulated point for the measurement of the magnitude of the negative potential (N_1) and positive potential (P_1) of subsequently measured distorted tracings. Since the experimental conditions concerning the sound input for each experiment were kept constant the delay time with the tracings were also expected to be constant (Fex, 1965). The experiments were conducted in an electrically shielded Industrial Acoustic Company Chamber Model 5A. Permanent records were filmed with a Grass C4-K Camera for later measurement. Paired one tailed *t*-test was performed comparing each group with its own control values using $P < 0.05$ as the acceptable level for indication of a significant difference.

Stimuli

A click stimuli was generated to excite the r.w. potentials. The click was generated in the TDH 39 earphone by a square wave (0.05 msec duration, 0.05 volts) placed across the terminals, generated by a Grass S8 stimulator. Fig. 4 shows the spectral peaks of the click with the measuring microphone in place of the animal's ear. The click, when generated in the earphone placed next to the left ear, with the ear bar removed, resulted in a reproducible cochlear

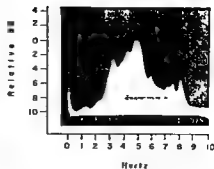


Fig. 4. The spectral analysis of the TDH 39 earphone output without an ear bar is shown. The earphone was repetitively stimulated with 0.05 V, 0.05 msec duration square wave pulse. The spectrograph was obtained with a 1/2 inch Bruel & Kjaer Condenser Microphone connected to a Tektronic Spectrum Analyzer 3L5 model. The peak output is slightly below 5 KHz with several lesser peaks through the spectrum.

potential complex. The intensity selected produced a tracing with a low amplitude microphonic tracing, and a prominent N_1 and P_1 potential (see Fig. 3). The intensity of the sound stimuli was not sufficient to cause elicitation of the middle ear reflex and was calculated to be a peak value of -26 dB re 1 Volt = 0.0 dB. The sound stimuli parameters and environment for each experiment were kept constant.

Histology

The placement of the lesions in the cochlear nucleus were identified by the use of histological preparations. The cat brain was perfused with saline at the end of the experiment and then fixed with neutral formaldehyde solution. The fixed brain was trimmed to a block containing the cochlear nucleus and embedded in Paraplast by the usual Autotechnicon procedure. Serial 10μ m sections were then stained with Hematoxylin and Eosin (see Manual of Histological and Special Staining Techniques, 1949) and mounted for light microscopy. The guinea pig cochlear nucleus were not studied histologically.

RESULTS

Measurements of N_1 and P_1 magnitudes after different lesions are presented in Fig. 2. Each

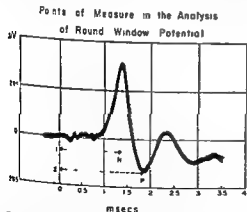


Fig. 3. This is a reproduction of a tracing obtained from the round window with the experimental system described in Methods. The time relationship used for measurement of the appearance of the peak of the negative potential (N_1) and the positive potential (P_1) in control tracings is indicated. These measurements (1.2–1.6 msec latency after the NC) were then used to locate the point of measurement to obtain the extent of the distortion of the P_1 potential produced by the various lesions.

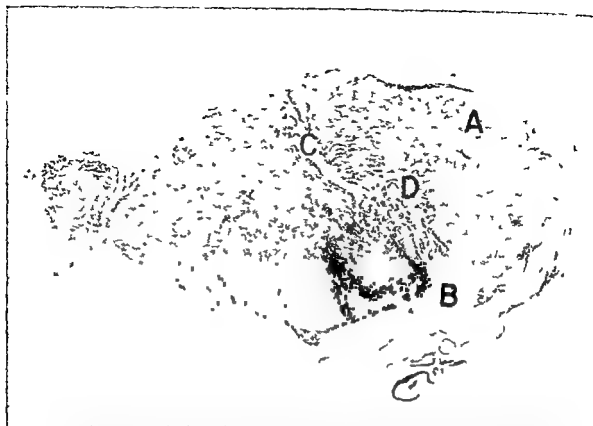


Fig 5 A parasagittal section through the location of the lesion placed in the peripheral portion of the cochlear nucleus. The lesion was produced with a 1 mm diameter electrode and a current of 2 mA for 15 sec. The tissue is

10 μ m thick stained with hematoxyline and eosin (A) dorsal cochlear nucleus (B) ventral cochlear nucleus (C) electrode pathway (D) lesion location Magnification is $\times 23$

lesion as depicted in Fig 1, interrupted the efferent innervation at various levels of its course, from the segment of the olivary nucleus to the cochlea. The lesions which produced a significant loss of the positive potential ($P < 0.05$) were those placed either in the vestibular portion of the eighth nerve (lesion 6) in cats, the VIII nerve complex in guinea pigs and in the lateral part of the dorsal aspect of the cochlear nucleus (lesion 5) in cat and guinea pigs. Lesions which interrupted the more central pathways either of contralateral or ipsilateral efferent fibers produced no changes associated with the positive wave (lesions 1, 2, 3 and 4).

Lesions placed in the lateral part of the dorsal cochlear nucleus (type 5) by either a wire section, phenol injection or electrical coagulation all produced the same initial loss of the P_1 potential (see Fig 2). These procedures do not affect the

N_1 latency nor do they significantly change its amplitude ($P < 0.05$).

The rate of P_1 recovery is different after various lesions. When lesions were placed at the level of the vestibular nerve (lesion 6) extensive recovery of the P_1 potential occurred in a period of 2 hours (Daigneault et al 1968). Phenol injections into the lateral 1/3 of the cochlear nucleus as observed from a dorsal exposure (lesion 5) also showed a similar degree of reorganization of the P_1 wave. In contrast with the electrical lesions placed in the same region the distorted potential remained the same during a 4 hour observation period.

The histological evidence for localization of the area of the cochlear nucleus responsible for the changes is presented in Fig 5. The location is in the area where the dorsal cochlear nucleus and the caudal portion of the ventral nucleus

peripherally. The cells at that location which would have been injured are small spherical cells and multipolar cells as described by Olsen (1969). Affrent fibers of the descending branch from the basal turn are found in this area of the cochlear nucleus of the cat and the monkey (Moskowitz & Liu 1972).

Similar experiments performed upon guinea pigs produced parallel results. Only lesions associated with the lateral 1/3 of the cochlear nucleus, as viewed from the dorsal surface, and the VIII nerve caused the distortion of the cochlear tracings. In our hands, it was not possible in the guinea pig to obtain the changed pattern with only vestibular nerve section. The acoustic nerve had to be included before the extent of distortion equaled that observed in the cat.

DISCUSSION

The proper utilization of round window tracings, for either research laboratories or as a human diagnostic procedure, will require accurate interpretations of the various potential components. This study extends our earlier work (Dauwout et al., 1968) which dealt with changes produced by vestibular nerve lesions upon the P_1 components of the *rw* tracings. From our experience with pharmacological experiments which have dealt with possible mediators or intoxicants of the cochlea we have found that the extent of possible interpretations of the tracings are limited. The present data indicate that the positive wave (P_1) is predictably affected by lesions placed peripheral to or at a specific part of the cochlear nucleus. The resulting changes in the electric pattern may be the result of changes produced in or near the cochlea. These may be either physical or neural changes induced by the lesion and reflected in the recording. Since some procedures produce either transient or prolonged (4 hours) changes in the *rw* pattern, the neural mechanism seems to be a plausible one.

As Rasmussen stressed by quoting Lorente de Nó (Rasmussen & Windle, 1965) it is most urgent that we obtain knowledge of minute struc-

ture to enable proper interpretation of physiological data. The clue that the P_1 portion of the system can be associated with a part of the cochlear nucleus and possibly an efferent innervation helps to guide the direction the study should take. By some mechanism it seems that a peripherally active system is responsible for an inhibition with a rapid onset of action (Matsubara et al., 1971). The situation which may be operating at this peripheral site may be either that neurons are interacting upon other neurons in the spiral ganglia or with those in the cochlear nucleus, or the neurons may be interacting with an efferent fiber which courses through the area. Since the characteristics of the P_1 indicate that this is a neural phenomenon with a short delay time, it may well indicate that one of these mechanisms is operating. The short delay time of this phenomenon places it as a possible candidate for the mechanism postulated by other investigators (Katsuki et al., 1962; Moushegian et al., 1971) that a peripheral inhibitory mechanism may well exist. A working example of this hypothesis would be that this is an active coding system present at this peripheral site accounting for signal processing as described by Lynn (Lynn & Sayers, 1970).

The characteristics of the N_1 portion of this tracing can be informative in the interpretation of these observations. The particular lesions which have been observed to produce changes in the cochlear potential (P_1) do not affect the latency of N_1 nor reduce the height of N_1 . The lack of N_1 depression is evidence that the procedure did not initiate the usual olivocochlear bundle activity. It is possible that we are observing a limited action of the OCB or the activity of a different neuron system. It is proposed that the P_1 potential is either a separate neural system not previously recognized or a system interrelated in some way with a different level of activity of the efferent system. It is hypothesized that this neural system is responsible for the generation of the positive potential which may be active as a peripheral inhibitory or coding system.

A point of information from past experiments which has been a challenge to explain is the

extent of recovery of the P_1 potential as a consequence of sectioning the olivocochlear bundle in the vestibular nerve. The persistent loss of P_1 , produced by the electrocoagulation of specific areas of the cochlear nucleus, may provide a clue towards obtaining an answer. From the experiments dealing with the selective section of the VIII nerve (Daigneault et al., 1970) it was evident that a minor portion of the fibers responsible for the P_1 potentials possibly exist in the acoustic nerve. When our experiments were designed so that these fibers within the acoustic nerve were intact and thus available, for the still functional elements in spiral ganglia and the cochlear nucleus, a prominent degree of recovery was accomplished. When a local anesthetic was used on the VIII nerve complex, recovery did not occur for the duration of the long-lasting local anesthetic (Dibucaine) (Daigneault et al., 1968). Therefore, when the elements responsible for the activity in the area of the cochlear nucleus are thus destroyed, or as with the local anesthetic, all the fibers are blocked for several hours, during that time the pattern of recovery does not occur. This may be indicating that a role is being played by the neurons of the cochlear nucleus and/or the spiral ganglia and these are associated with the area to be considered as the generator of the positive potential. It will be necessary to further explore the anatomy of this area in future experiments to verify if these assumptions are correct. Such experiments utilizing light and electron microscopy are currently in progress in our laboratory with our attention directed to the spiral ganglia (Adamo & Daigneault, 1973).

With the development of procedures to obtain cochlea potential recordings from patients by using signal averaging techniques, the application of this technique in clinical work is being investigated. The descriptive data of Aran (Aran et al., 1969) obtained from various patients with and without pathological conditions, have shown a wide variety of patterns. The normal patterns are very much in agreement with the tracings obtained from the cat or guinea pig. The abnormal patterns associated with pathological

conditions are in most cases not interpretable. From personal communications with Dr Aran, published reports of Salomon & Elberling (1971) and similar recordings at our facility (Culle et al., 1972) some abnormal patterns similar to those reported in this investigation have been observed from patients. It is reasonable to expect that other examples of correlation between location of pathology and the alteration of the shape of the cochlear potentials may be found in the future. This type of information may make the human cochleogram a powerful diagnostic tool.

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The assistance of Dr C. Berlin of the Gulf South Research Foundation in obtaining the spectral feature of the TDH39 earphone and the excellent laboratory workmanship of Mr Carlos Devia and Mr Clarence Foster Jr are greatly appreciated.

ZUSAMMENFASSUNG

Katzen und Meerschweinchen wurden benutzt um den Einfluss spezifisch lokalisierter Nervenläsionen auf die durch ein kurzes Klickgeräusch hervorgerufenen Aktionspotentiale des Fenestra rotundum zu untersuchen. Die Zerstörung eines spezifischen Gebietes des Nucleus cochlearis durch Elektrokoagulation führte zu einem Ausfall der positiven Phase des Potentials. Das Potential normalisierte sich während einer Beobachtungszeit von vier Stunden nicht, im Gegensatz zu der weitgehenden

enthalt) angesetzt wurde. Die mangelnde Reversibilität bei Tieren mit Läsionen im Nucleus cochlearis kann damit erklärt werden, dass die verantwortlichen Neuronen dieses Gebiets zerstört worden sind. Da das P_1 Potential eine kurze Latenz im Verhältnis zur Ausdehnung der mikrophonischen Aktivität hat und da die Veränderungen nur auftreten, wenn die Läsionen in der Peripherie vom oder im spezifischen cochlearen Kerngebiet angesetzt werden, kann gefolgert werden, dass das P_1 Potential die Aktivität eines peripheren Coding oder Hemm Mechanismus darstellt.

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ELECTRONYSTAGMOGRAPHY ON NORMAL PERSONS

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Abstract Twenty normal persons were studied by electronystagmography (ENG) for spontaneous positional and caloric nystagmus. In addition a pendulum test was performed. Spontaneous nystagmus was demonstrated in 3 persons. Another 6 had positional nystagmus in 1-4 positions with a maximum angular velocity of 7.5°/sec. The parameters (duration frequency and angular velocity) after caloric stimulation were analyzed statistically. The duration parameter showed least relative standard deviation. There was no sign of a correlation between sex age, and the data measured. No abnormal curves were found in the pendulum test. On the basis of examples of centrally and peripherally conditioned nystagmus the pendulum test appears well suited for distinguishing between these types of nystagmus.

By electronystagmography (ENG) it is possible to objectify spontaneous nystagmus (SN) or provoked nystagmus and to describe the parameters of the ENG curve. In order to assess the value of such studies it is of decisive importance to be in possession of comparable studies on normals. The present investigation was designed to present an electronystagmographic diagnostic programme and to assess the results of tests on normal persons.

MATERIAL AND METHOD

The material consists of 20 persons of an age distribution and sex ratio corresponding to the patients examined by ENG in the Department within a 5-year period (Fig. 1). All the subjects were otologically normal, had no complaints of dizziness or hearing impairment and were on no kind of medication.

The recording was done as conventional electronystagmography, using a 4-channel writer

Type ELEMA Mingograf 34 The mingograph was calibrated so that an ocular deviation of 10° corresponded to an excursion of 20 mm on the curve. The subjects were tested after 30 minutes' adaptation to the dark in order to obtain a stable corneal retinal potential (Munthe-Fox 1963). They were investigated for SN and positional nystagmus (PN) and had caloric test as well as a pendulum test. The examination was carried out in a dark room and the subjects had their eyes open and wore dark spectacles except in the pendulum test. By this means we avoid inhibition of nystagmus due to ocular fixation and obtain at the same time better registration of nystagmus with open eyes. To counteract voluntary inhibition of the nystagmus the subjects were asked to perform subtraction (1 000-7) during the test. In the pendulum test the subject follows with his eyes a swinging pendulum in a dim red light.

At the conclusion of the test it was checked whether the calibration had remained constant. As stimulus in the caloric test we used irrigation with water at 44°, 250 ml for 30 sec. The irrigation was done by an automatic temperature- and time regulated irrigation system (Type 06 C 643 K). As stimulus we used a better stimulus than the one used by Lowenstein (1959) among others. By omitting the

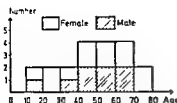


Fig 1 Age-distribution and sex ratio of the material

diagnostic procedure is simplified, and the rare cases of pseudocaloric nystagmus are avoided (Greisen, 1972)

Since the various ENG parameters following caloric stimulation are attributed with different validity, and since their numerical calculation is different, we analysed statistically and compared the following parameters in our 20 normal subjects

- I Maximum angular velocity of the slow phase which is induced by caloric action, whereas the rapid phase is a central compensation phase
 - II The number of nystagmic beats between 60 and 90 sec after starting the stimulus, as empirically this is the time interval in which the angular velocity of the slow phase as well as the frequency are at a maximum
 - III Total duration of caloric nystagmus measured in seconds
- Moreover, the difference between the right and left side in each person within the 3 parameters was calculated

The pendulum test was carried out by letting a recumbent subject follow with his gaze a swinging pendulum placed above the subject while simultaneously the ocular movements were recorded as usual in ENG. The pendulum consists of a small steel ball suspended in a long, thin string, giving a swinging time of about 2 sec. The distance of the pendulum from the person is about 40 cm. Normally, the pendulum is made to swing horizontally and/or vertically in relation to the subject, with an excursion corresponding to 15–20° rotation of the eyes. According to Maspétiol et al (1967) and Fonsman & Johansen (1971) normal persons and patients with peripherally conditioned nystagmus exhibit a regular

sinus curve when watching the swinging pendulum, whereas a patient with centrally conditioned nystagmus shows an irregular sinus curve. Thus, by this simple test it ought to be possible to distinguish between peripherally and centrally conditioned nystagmus

RESULTS

Of the 20 normal persons, 3 had SN with 3.5°/sec of the slow phase as the maximum value. The recorded SN was largely unchanged on examination in various positions. The 3 subjects having SN were of the age groups 20–29, 50–59, and 60–69 years. Another 8 subjects had PN in from 1 to 4 positions. The maximum angular velocity of the slow phase was 7.5°/sec. These cases were distributed on all age groups.

The recorded data following caloric stimulation for the maximum angular velocity of the slow phase—number of nystagmic beats between 60 and 90 sec as well as the duration measured in seconds—were statistically analysed (with assistance from the Department of Data Processing) by Wilcoxon's signed rank test, acting on the hypothesis that the distribution is symmetrical around the mean value, and by the Kolmogorov-Smirnov test for comparison with the normal distribution having the same mean value and standard deviation. By these tests it was not possible to demonstrate a deviation from the normal distribution. It was only concerning certain data for the maximum angular velocity of the slow phase that some doubt arose as to whether the distribution was normal. It must be mentioned, however, that the size of the data does not afford the best possibilities for disclosing deviations from a normal distribution by statistical analysis. If a transformation of the maximum angular velocity data was performed by calculating 10 × the logarithm of these values (Hinchcliffe, 1967), these logarithmic data were of a normal distribution with a smaller Kolmogorov-Smirnov fractile than the original data. This is interpreted as an improved adaptation to a normal distribution. When analysed in this way, the present material gave values practically

Table I *Statistical results of the single side values (caloric test)*

Single side values Caloric (44°)		Wilcoxon fractile	Kolmogorov Smirnov fractile	Common mean	Common S D	Relative deviation	Fractile	
							2.5%	97.5%
Speed of slow phase	R	0.521	0.941	23.9	16.29	68.2%	-8.7	56.5
	L	0.277	0.343	*/sec	*/sec		*/sec	*/sec
10 × log speed of slow phase	R	0.234	0.332	13.01	2.58	19.85%	6.1	65.6
	L	0.629	0.336				*/sec	*/sec
Number of beats (60-90 sec)	R	0.774	0.830	76.1	23.75	31.25%	28	123
	L	0.103	0.136				beats	beats
Duration	R	0.014	0.173	176.23	31.87	17.9%	112	240
	L	0.074	0.553	sec	sec		sec	sec

coincident with those of the Hinchcliffe material. Furthermore, it was not possible to demonstrate statistically a significant difference between right and left values, and no correlation between sex, age, and the measured data could be found.

From a statistical point of view it is expedient to select the parameter which has least relative standard deviation (the narrowest Gaussian curve) to express the normal range. The results are apparent from Table I. It is apparent that the duration shows the least relative standard deviation of 17.9%. If the duration in a normal material is plotted on probability paper, giving the mean value as well as the 2.5% and 97.5%

we get a straight line, from which it is possible to estimate with how much probability a measured value on one side is normal. As may be seen from Table I, the 2.5% and 97.5% fractiles for duration were 112 sec and 240 sec respectively and the mean value 176 sec (Fig. 2). Thus, values below 112 sec indicate hypofunction, whereas values above 240 sec

indicate hyperfunction (SN and/or directional preponderance (DP)).

The measured data for the three parameters show a marked difference between the values from one person and those of another within the individual parameter—and also in some cases a marked difference between the values for the right and left side in the individual subject. Since theoretically it would be expected that in normal persons the difference between the values for the right and left side in each individual is 0 or close to 0, it seems natural to express the difference between the right and left side in per cent of the two sides' numerical sum value as the background of the above mentioned marked individual difference. Something like this was suggested by Jongkees et al. (1962) among others. Since, moreover, it is desired that the difference values expressed in per cent should—if possible—be of a normal distribution the differences have to be calculated with opposite signs in order to obtain a symmetrical distribu-

Table II *Statistical results of the difference values calculated in per cent (caloric test)*

Difference values in % Caloric (44°)	Wilcoxon fractile	Kolmogorov Smirnov fractile	Mean	S D	Error of mean	Fractile	
						2.5%	97.5%
Diff. speed of slow phase	0.462	0.125	7%	19%	±11%	45%	+31%
Diff. number of beats	0.474	0.243	3%	11%	+6.3%	25%	+19%
Diff. duration	0.729	0.566	6%	5%	+4.6%	22%	+10%

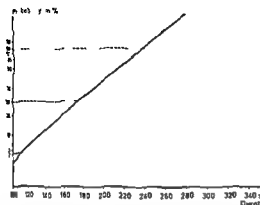


Fig 2 Duration (single side values) of the caloric tests (44°) plotted on probability paper. The middle dotted line represents the mean value—the upper and lower lines represent two times the standard deviation

tion around the mean. Arbitrarily, we decided to call the right side values positive and the left side values negative. This calculation showed that the differences fitted well in with a normal distribution. The results are presented in Table II. Here too, it is evident that the duration parameter for the difference values gives least standard deviation (8%). In a way similar to that used for the values for each side, the difference values may be plotted on probability paper as a straight line with the 2.5% and 97.5% fractiles (Fig 3). Thereafter, the normality of calculated differences may be immediately estimated by plotting on the curve.

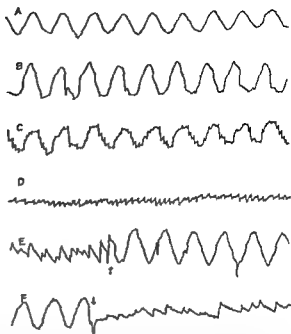
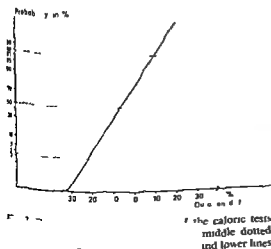


Fig 4 Pendulum test (A) Regular sinus curve (B) Slightly irregular sinus curve (C) Serrated abnormal pendulum curve from a patient with epilepsy (D) SN from the same patient as C (E) and (F) show caloric induced nystagmus disappearing when the eyes follow the pendulum and re-appearing when the eyes are closed

Of the 20 normal persons 17 had a regular sinus curve in the pendulum test, as shown in Fig 4A. Maspétiol et al (1967) distinguish between a normal, regular sinus curve and three different types of abnormal sinus curves. In the present paper a distinction is made between a normal, regular sinus curve, a slightly irregular sinus curve, and an abnormal sinus curve. Three out of the 20 normal persons had a slightly irregular sinus curve of the type shown in Fig 4B. Two of these 3 subjects had SN and the third had PN.

DISCUSSION

The reported findings of SN and PN in ENG of normals have differed. For instance, Stahle (1958) in a series of 50 normals, found none to have either SN or PN, and Jongkees et al (1962) detected SN in only one of 85 normal subjects (angular velocity not stated). According to several authors only a SN over a certain angular

velocity can be considered abnormal Bos et al (1963) stated that a SN exceeding 6°/sec angular velocity was highly suggestive of an abnormal condition, and Hinchcliffe (1967) found, among 20 normal persons, 2 who had SN, in both of them below 6°/sec angular velocity. In the present material 3 persons had SN, though not exceeding 3.5°/sec angular velocity. Another 6 had PN, maximum angular velocity 7.5°/sec.

To assess an abnormal caloric reaction it is important to know the values for each side as well as the difference value, as these values supplement each other. For instance, it is not always possible to conclude from an abnormal difference value which side, or whether both sides give an abnormal caloric reaction. As is evident from Table II, all the mean values for the various difference parameters were negative, although they would be expected to be 0 or close to 0. Against this background an investigation was made for faults in the apparatus or procedure, but no such faults were found. The phenomenon may be due to the relatively small material (cf the statistical error of the mean, Table II) or the fact that the right ear is always irrigated first. This possibly entails a psychic inhibition due to fear of the unknown, a factor which is perhaps not present at the subsequent irrigation of the left ear.

Since it was to be expected, *inter alia* on the basis of Lowenstein & Sand's study (1940), that in normal individuals there is a vestibular resting tonus, the vestibular apparatus of the two sides keeping each other in equilibrium, it is surprising to observe how marked a difference there may be between the caloric values for the two sides in the same normal person. In view of the fact that the externally administered caloric stimulus is technically and physically identical on both sides, the marked difference between the two sides is probably due to the caloric energy administered to the semicircular canals being different nevertheless. As shown by the values, the caloric test must be considered a relatively rough test.

On the basis of an analysis of a normal material, the duration parameter seems most expe-

dient. From this it is not permissible to conclude direct that this will also apply to an abnormal material. It is only by applying the parameters to such a material that it is possible to decide which is most expedient. The duration—estimated on the basis of the ENG curves—posed no problem.

The pendulum test does not represent a reflex, as does the caloric nystagmus, or a partial reflex, as does optokinetic nystagmus, but is a purely voluntary act, presupposing that the patient can see and follow the pendulum with his eyes. This test showed that normals have either an entirely regular sinus curve or a slightly irregular one. Those having a slightly irregular sinus curve had either a faint SN or a PN.

Fig. 4C presents an abnormal—serrated—asymmetrical sinus curve from a patient showing signs of epilepsy. Fig. 4D shows the registration of SN from the same patient as the one in Fig. 4C. It is obvious that the SN manifests itself on the sinus curve.

Fig. 4E represents the same normal subject as Fig. 4A. The first part of the curve shows a caloric reaction after irrigation of the right ear with 44° water for 30 sec. At [1] the eyes were opened, whereupon the eyes followed the movements of the pendulum, subsequently giving a regular sinus curve without any manifestation of the marked caloric nystagmus.

Fig. 4F is the continuation of E—after a regular sinus curve of several seconds' duration. At [2] the subject again closed his eyes, and thereafter the normal nystagmus was again manifest on the ENG curve.

In deciding whether a patient has a vestibular or a central disorder, many authors base their deductions on the caloric reaction, a weakened caloric reaction often being interpreted as a sign of peripheral disease. Studying midline lesions in the brain stem of cats Stroud et al (1971) demonstrated that such lesions may temporarily as well as permanently abolish the caloric reaction. This, then, can hardly be a consistent indication of a peripheral disorder.

The result of the pendulum test on normals, which revealed no abnormal curves, as well as

the examples shown in Fig 3 indicate that the pendulum test is an important aid in deciding whether nystagmus is of central or peripheral origin

ZUSAMMENFASSUNG

Zwanzig normale Menschen wurden mit Elektronystagmographie auf Spontan-, Lage- und kalorischen Nystagmus hin untersucht. Daneben wurde ein Pendeltest ausgeführt. Spontan-nystagmus wurde bei 3 Personen festgestellt. Weitere 6 Personen hatten Lagenystagmus in 1-4 Positionen, wobei die Winkelgeschwindigkeit maximal 75/sec betrug. Die Parameter (Dauer, Frequenz und Winkelgeschwindigkeit nach der kalorischen Stimulation) wurden statistisch analysiert. Der Dauerparameter zeigte die geringste relative Standardabweichung. Es bestand kein Zusammenhang zwischen Geschlecht, Alter und den gemessenen Daten. Im Pendeltest wurden keine abnormen Kurven gefunden. Aufgrund von Beispielen an zentralen und labyrinthar vestibulären Nystagmen wird angenommen, dass der Pendeltest zur Trennung von diesen Typen von Nystagmus geeignet ist.

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THE EFFECT OF VISUAL INPUT ON POST-ROTATORY NYSTAGMUS IN NORMAL CHILDREN

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Abstract The duration, total number of beats, and frequency of post rotatory nystagmus were measured in normal young children under different conditions of visual input. After rotation in darkness, the effects of fixation without light, fixation in light, and light without fixation on the nystagmus evoked by an abrupt braking deceleration were studied. The post rotatory nystagmus duration and the total number of beats were significantly reduced both by fixation without light and by light without fixation. The findings were discussed in relation to mechanisms by which vestibular nystagmus may be suppressed.

The suppressive effect of visual input on vestibularly-induced nystagmus has been well documented for adult subjects (Wendt, 1951, Collins, 1966, Marshall & Brown, 1967, Collins, 1968, Hood, 1970, Pfaltz & Piffko, 1970).

studies have not been reported for

In the adult studies, visual fixation has been assumed to be the effective mechanism by which vestibular nystagmus is suppressed in the presence of visual input, however, the relative influence of fixation *per se* and the effect of light on the vestibulo-ocular reflex has not received comment. Interest in the present investigation in normal children was generated by an earlier finding that the suppression of post-rotational nystagmus in autistic children (Pollack & Krieger, 1958, Colbert et al., 1959) was dependent on a free visual field and did not occur in dark-

ness (Ritvo et al., 1969). In preparation for further studies of the interaction of visual and vestibular stimuli in autistic and other developmentally deviant children, this investigation explores the relative contributions of visual fixation and light to suppression of post rotatory nystagmus in normal children.

METHODS

Subjects

Twenty-five 38-90 month-old (mean 56.4 months) normal children were studied for 3 sessions each. The sessions occurred at 2 to 4 day intervals. Sixteen were boys and 9 were girls.

Stimulation

In order to obtain data that would be comparable to that obtained in the studies of autistic children (Pollack & Krieger, 1958, Colbert et al., 1959, Ritvo et al., 1969), the conventional Barany procedure of 10 complete revolutions in 20 sec followed by an abrupt stop was employed. The subjects sat in an electromechanically driven and remotely controlled chair which was rapidly accelerated from rest to 180°/sec in complete darkness for 20 sec. The horizontal semicircular canals were maintained in the horizontal plane by partial fixation of the subject's head with a padded occipital brace and an elastic head strap. Subjects received 6 clockwise and 4 counterclockwise rotations during each

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Table I *The experimental conditions used to study the influence of visual input on post-rotatory nystagmus*

Condition	Purpose
1 Standard visual field with standard illumination	To determine the degree of suppression of nystagmus in light when fixation is possible but is not encouraged
2 Standard visual field with fixation object added	To determine if nystagmus suppression would be enhanced when deliberate fixation is encouraged
3 Pinpoint of red light with minimal light value in complete darkness	To study the effect of fixation in the absence of light
4 Goggles admitting light without visual pattern. Fixation precluded. Light intensity equivalent to Condition 1	To study the effect of light without fixation
5 Same as Condition 4, but with lower light intensity	To study the effect of light intensity
6 Complete darkness	For comparison with other conditions. This is the 'neutral' condition

session. At least 4 minutes elapsed from the end of one rotation to the beginning of the next. Each pair of clockwise and counterclockwise rotations in absolute darkness was immediately followed by one of the visual conditions outlined in Table I. In Condition 1, the chair always stopped facing an evenly illuminated white background. The children received no special instruction other than to keep their eyes open and avoid head movements. In Condition 2, a black cross was added to the white background directly in the line of vision when the chair stopped. The children were encouraged to stare at the cross. In Condition 3, they were encouraged to look at a pinpoint of red light directly in the line of vision when the chair stopped; the room remained absolutely dark and the point of red light provided no illumination. In Condition 4, the subject wore frosted goggles while rotating in the chair. When the lights came on, a bright white patternless field was visible but fixation was precluded. Condition 5 was similar except that the illumination was less: the subjects saw a dull gray patternless field. In Condition 6, the room remained absolutely dark after the chair stopped. In Conditions 1 through 5, the various types of light came on at the exact moment that the chair abruptly stopped. Condition 1 was always the first and Condition 6 was a

last condition during each of the 3 sessions. The sequence of Conditions 2-5 were varied during the 3 sessions to permit assessment of the effects of order of presentation.

An adult was in the room with the child at all times but was out of the child's line of sight when the chair stopped. The adult could "see" any head movement in the dark during rotation by observing the relative position of two pin points of red light (not visible to the child) attached to the side of the child's head.

Recording

Horizontal eye movements were recorded bipolarly from silver-silver chloride electrodes at the outer canthi on a Grass polygraph using a paper speed of 10 mm/sec. The d.c. coupling was modified so that a 3 sec time constant was induced. Vertical eye movements and blinks were recorded on a second channel from leads placed directly above and below one eye.

Data sampling

It was necessary to discard a certain trials because of (1) lack of a child, (2) drowsiness or falling asleep, (3) movement during or after to keep eyes open, and (5) fati

Most of the children cooperated very well but occasional trials were discarded because, e.g., the child brought his hand up to his face, talked during the trial, etc. Trials during which drowsiness or sleep occurred were discarded. In addition to visual observation of the child when lights were on, drowsiness was determined by observing an absence of blinking combined with slow rolling eye movements on the electro-oculogram (EOG). The absence of blink artifact was also used to determine that the child was not keeping his eyes open. Trials were discarded whenever head movements were observed.

Those trials in which Conditions 2 through 5 obtained (see Table I) were subjected to additional scrutiny. Three of the investigators independently examined the EOGs for these conditions without knowledge of the age or identity of the subject to determine if ocular fixation did or did not occur. A trial was discarded if it was determined that ocular fixation had occurred under Conditions 4 or 5 or had not occurred under Conditions 2 or 3. If the three investigators did not unanimously agree to discard or retain a trial, a final decision was reached after discussion. If consensus could not be reached, the trial was also discarded. The presence or absence of fixation was determined by evaluating the relative amplitude of the nystagmus.

equated fixation during Conditions 2 and 3 always associated with nystagmus amplitude which was clearly smaller than that of Condition 4. The presence of undesirable fixation during Conditions 4 and 5 was associated with a nystagmus amplitude which was similar to that of Conditions 2 and 3.

Twenty-five of the scheduled 900 trials (25 subjects \times 3 sessions \times 6 conditions \times 2 directions) could not be carried out due to lack of subject cooperation (21 of these involved Conditions 4 and 5, the children refusing to wear the goggles). Forty-five extra trials were carried out in addition to the 900 scheduled trials, making a total of 920 trials actually run. Of these 920 trials, 137 were ultimately discarded for one or more of the reasons described above, leaving 783 successful trials for data analysis.

Table II *The relative success in carrying out the different experimental conditions in children*

	Number of trials attempted	Number of successful trials	Percent of successful trials
Condition 1	154	148	96.1
Condition 2	153	133	86.9
Condition 3	167	136	81.4
Condition 4	152	112	73.7
Condition 5	140	109	77.9
Condition 6	154	145	94.2
Total trials all conditions	920	783	85.2

The majority of the 137 unsuccessful trials (83 trials) were discarded because the criteria for presence or absence of ocular fixation were not met. Table II gives a measure of the degree of success in carrying out the different experimental conditions in young children.

Data analysis

Both the duration of nystagmus and the number of nystagmic beats following the abrupt stop of the chair were measured.

Two measures of duration were used. These measures were developed during a preliminary phase of this investigation to take into account the variability of children's nystagmograms. The "uninterrupted" duration score is the duration in seconds from the end of rotation to the last nystagmic beat of a train of beats in which the time between the end of one beat and the beginning of the next does not exceed 1.0 sec.¹ The "total" duration score is the duration in seconds from the end of rotation to the end of the last nystagmus beat of a series of 3 consecutive beats regardless of earlier disruptions in the nystagmogram.

Two measures of the number of beats were

¹ A nystagmic beat is defined as a slow phase immediately followed by a fast phase. Two qualifications of the definition are: (1) the slow phase must be longer than 1.0 sec; (2) the fast phase must be longer than 1.0 sec.

nystagmic beats longer than 1.0 sec was accepted as a train of beats continued after the disruption for a period of time greater than the sum of the time of the disruption and the previous period of nystagmus.

Table III Group means and standard deviations of nystagmus durations for 22 normal children under 6 conditions of visual input

Condition	Uninterrupted duration (sec)				Total duration (sec)			
	Both directions		Maximum direction		Both directions		Maximum direction	
	\bar{x}	$\pm S D$	\bar{x}	$\pm S D$	\bar{x}	$\pm S D$	\bar{x}	$\pm S D$
1	3.83	2.07	4.98	2.38	4.57	2.72	6.08	3.32
2	4.66	2.04	5.84	2.68	5.35	2.42	6.63	3.03
3	9.50	3.20	10.85	3.79	10.93	3.54	12.62	4.11
4	13.21	4.22	15.17	5.06	16.95	6.06	20.01	7.42
5	12.04	4.28	14.79	5.27	16.50	5.11	19.57	6.31
6	22.15	5.20	25.61	5.72	26.04	6.10	29.46	6.76

used (1) the total number of beats following the end of rotation, and (2) the frequency of beats (the number of beats per each 3 sec interval following the end of rotation). For the latter measure, if artifact obscured part of a 3 sec interval, that interval was treated as missing data in subsequent analyses.

For each condition, two methods were used to summarize the data obtained from a maximum of 6 trials (one pair of CW and CCW trials per session for 3 sessions). The first was to take the mean of all successful trials. Because of the possibility of directional preponderance, a second method was to calculate the mean of the maximum values from the pairs of CW and CCW trials from each session. Since both methods yielded similar results, only the results using the first method will be reported in detail.

RESULTS

Directional preponderance

To determine whether or not directional preponderance was present, χ^2 goodness of fit tests were applied to all usable pairs of clockwise (CW) and counterclockwise (CCW) trials for each of the 25 children using the uninterrupted duration scores.¹ The number of pairs of trials in which CW - CCW was compared with those

in which CCW > CW for each child χ^2 with 1 degree of freedom was greater than 3.84 ($P < 0.05$) for only 4 of the 25 children. To obtain a simultaneous test for the group of subjects, χ^2 and degrees of freedom were summed for the 25 subjects giving $\chi^2 = 40.14$ which was significant ($P = 0.05$). However, over 50% of the χ^2 for the group of children was accounted for by only 4 subjects.

Habituation and the order of presentation of trials

The possibility of habituation across the 3 experimental sessions was tested by comparing the mean nystagmus durations from each session for each of the 6 conditions between the pairs of sessions 1 and 2, 1 and 3, and 2 and 3 (t -test on paired dependent observations two-tailed). For both the uninterrupted and the total duration scores, there were no consistent changes across the 3 sessions. In fact, only one t test was significant which is expected due to the number of tests performed.

Similarly, computation of both the total number of beats and the frequency of beats revealed no clear pattern of habituation. More paired t tests were significant, but most of these differences lost significance when a multiple range test was used to take into account the comparison between three days.

Since the order of Conditions II, III, and IV was varied on the three days, the

¹ Data were available for both the CW and CCW direction for 8 to 18 pairs of trials (mean 14.0 pairs) out of 11 possible pairs of trials.

Table IV Group means and standard deviations of number of nystagmus beats for 22 normal children under 6 conditions of visual input

Condition	Total number of beats				Frequency of beats (beats/sec)			
	Both directions		Maximum direction		Both directions		Maximum direction	
	\bar{x}	$\pm S D$	\bar{x}	$\pm S D$	\bar{x}	$\pm S D$	\bar{x}	$\pm S D$
1	72	40	138	76	12	03	17	05
2	121	55	196	86	18	03	24	04
3	259	80	394	124	21	04	26	05
4	261	109	360	134	15	03	19	03
5	251	99	350	130	14	04	17	05
6	390	135	488	160	14	04	18	05

consistent effects of the order in which conditions were presented within days

Age

For the group of 25 children, correlation coefficients were computed between age and the mean nystagmus durations for the uninterrupted and total duration scores for each of the 6 experimental conditions. There were no significant correlations between subject age and nystagmus response under any of the experimental conditions. Similar results obtained for the total number and frequency of beats except that there was a very slight tendency for the total number beats to increase with age in Condition 6 (complete darkness) ($r = +.40$, $P = 0.05$, two tailed).

Sex

The mean uninterrupted nystagmus durations, the total number of beats, and the frequency of beats from all successful trials of the 16 boys were compared with those of the 9 girls (two sample t tests with separate variances) for each experimental condition on each of the three experimental days. There were no significant sex differences which were consistent across the 3 experimental days.

The effect of the visual input condition

Since there was no habituation across the 3 experimental days and since order of presentation of conditions and sex and age of subject

did not influence the nystagmus scores, all successful trials were average for each subject in each condition. Twenty two of the 25 children had successful trials in all 6 experimental conditions.

Nystagmus duration A two-way analysis of variance (ANOVA) was carried out across these 22 subjects and 6 conditions. The ANOVA demonstrated that the conditions differed significantly ($P < 0.001$). Table III shows the nystagmus durations for the different experimental conditions. The Newman-Keuls Multiple Range Test was used to test the significance of differences between the different conditions. The uninterrupted post rotatory nystagmus durations were significantly longer in complete darkness (Condition 6) than in all other conditions ($P < 0.01$). Under both conditions of light without fixation (Conditions 4 and 5) and fixation without light (Condition 3), the nystagmus durations were significantly longer than in the two standard visual field conditions (Conditions 1 and 2) ($P < 0.01$). During fixation without light (Condition 3), the nystagmus durations were significantly shorter than during both light without fixation conditions (Conditions 4 and 5) ($P < 0.01$). The results were the same for the total durations.

Number of nystagmus beats

Two-way ANOVAs also demonstrated that both the total number and the frequency of beats differed significantly under the 6 experimental

conditions Table IV shows the total number and frequency of beats for the different conditions. Application of the Newman-Keuls Multiple Range Test showed that the results were essentially the same for the *total number of beats* as for the *duration scores*. There were significantly more beats in complete darkness (Condition 6) than in all other conditions ($P < 0.01$) and there were significantly more beats in Conditions 3, 4, and 5 than in Conditions 1 and 2 ($P < 0.01$). Unlike the duration scores, there was no significant difference in number of beats when Condition 3 was compared with Conditions 4 and 5.

The results of the analysis of the *frequency of beats* were substantially different. Those conditions which encouraged ocular fixation (Conditions 2 and 3) were associated with a greater nystagmus frequency than all other conditions ($P < 0.01$). There was a higher frequency with fixation in the dark (Condition 3) than with fixation in the light (Condition 2) ($P < 0.05$).

Correlation coefficients were also computed between experimental conditions for the 22 children who had successful trials in all 6 visual input conditions. For the *uninterrupted durations*, there were no significant correlations between complete darkness (Condition 6) and the other 5 conditions ($r \leq +0.32$). The nystagmus durations under the 3 conditions in which visual fixation was possible (Conditions 1, 2, and 3) were all highly correlated with each other ($+0.63 \leq r \leq +0.83$, $P < 0.01$) and were independent of the conditions in which visual fixation was precluded (Conditions 4 and 5) ($r \leq +0.23$). The latter 2 conditions were highly correlated with each other ($r \geq +0.55$, $P < 0.01$). Similar results obtained for the *total duration scores*, except that Conditions 1 and 3 were less strongly correlated ($P < 0.05$). Similar results also obtained for the *total number of beats*, except that Condition 6 was highly correlated with Conditions 4 and 5 ($r > +0.78$, $P < 0.01$).

DISCUSSION

The main finding of this investigation is that a group of normal children show a significant

reduction of post rotatory nystagmus duration and total number of beats under conditions in which visual fixation is precluded but in which light impinges on the retina. The suppression of nystagmus duration is not as profound as that induced by visual fixation in the absence of light input, but the suppression of the total number of beats is the same in light without fixation as during fixation without light. The greatest suppression of post-rotatory nystagmus occurs in those conditions which involve the influence of both fixation and light. Thus fixation and light appear to have an additive effect in the suppression of vestibular nystagmus. This effect does not depend on a positive correlation between the separate responses to light and fixation since the correlation analyses demonstrated that the subjects' responses to conditions permitting fixation were independent of their responses to conditions permitting light input but precluding fixation.

There have been almost no systematic studies of vestibular nystagmus in normal children. Allowing for differences in stimulation parameters, our post-rotatory nystagmus durations and frequencies in the dark are reasonably similar to the post-acceleratory nystagmus durations and frequencies reported by Tibbling (1969) for the same age group.

While the nystagmus duration and the number of beats give an incomplete measure of vestibular activity (Tibbling, 1969), the post-acceleratory nystagmus duration does reflect the intensity of the stimulus (Fluur & Mendel, 1969). In the present investigation both the nystagmus duration and the total number of beats can be considered measures not of the total nystagmus intensity but rather of the *persistence* of nystagmus following an abrupt deceleration under the different conditions of visual input. The results pertaining to the *frequency* of nystagmus beats help to understand the nature of the suppression of nystagmus duration and total number of beats in the presence of retinal stimulation with light. The nystagmus frequency was significantly greater during the two conditions which encouraged ocular fixation than during

conditions, and there were no differences in frequency during the light without fixation conditions and complete darkness. Greater frequency is a result of smaller slow phase amplitude or greater slow phase velocity or both. While these parameters were not quantified in this study, it was clear from inspection of the nystagmograms that during ocular fixation (Conditions 2 and 3), slow phase amplitude was severely depressed. This was not the case during light without fixation (Conditions 4 and 5) when the amplitudes tended to be similar to those recorded in complete darkness (Condition 6). Consequently, the frequency data verify the fact that the reduced persistence of nystagmus during the light without fixation conditions is not related to ocular fixation as is the case when a fixation object is provided.¹ This suggests that vestibular nystagmus can be suppressed by at least two relatively independent mechanisms: (1) a mechanism that is primarily oculomotor and does utilize ocular fixation, and (2) a mechanism that is primarily ocular sensory and does not utilize ocular fixation.

The former mechanism may be related to inhibitory cerebellar influences (Wolfe, 1969). The latter mechanism, in which light *per se* impinging on the retina causes the suppression of vestibular nystagmus, is obscure. However, ocular control of the vestibular nuclei is vital to the generation of nystagmus in response to vestibular stimulation (Pompeiano, 1972). Also, light evoked discharges from lateral geniculate nucleus neurons can be altered by vestibular stimulation and connections from the vestibular nuclei to the reticular formation and from the reticular formation to the lateral geniculate nuclei have been described (Papajohnnou, 1973). Therefore, it seems possible that mechanisms may exist whereby the effect of light on vestibular nystagmus is mediated by the reverse pathway, i.e. from the lateral

geniculate nuclei via the reticular formation to the vestibular nuclei. Such a mechanism would provide additional stability of visual perception during motion.

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ZUSAMMENFASSUNG

Die Dauer, Gesamtzahl der Schläge und Frequenz des Postrotationen Nystagmus wurden an normalen jungen Kindern unter verschiedenen Zuständen der visuellen Leistungsaufnahme gemessen. Nach der Rotation im Dunklen wurde die Wirkung von Fixierung ohne Licht, Fixierung im Licht und Licht ohne Fixierung auf den durch plötzliche Bremsverlangsamung hervorgerufenen Nystagmus studiert. Die Dauer des Postrotationen-Nystagmus und die Gesamtzahl der Schläge wurden durch Fixierung ohne Licht und durch Licht ohne Fixierung bedeutend vermindert. Diese Resultate wurden an Hand des Mechanismus mit dem vestibulärer Nystagmus unterdrückt wird besprochen.

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¹ Parenthetically, it can be seen that these young children did not fixate very well in Condition 1 when fixation was possible but not encouraged by a fixation object. The frequency in Condition 1 was not greater than in Conditions 4, 5, and 6.

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MALIGNANCY IN PLEOMORPHIC ADENOMA

A Clinical and Microspectrophotometric Study

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Abstract A clinical study of carcinoma in pleomorphic adenoma based on the most extensive material described in the literature hitherto, strongly indicates that a malignant transformation of a primarily benign pleomorphic adenoma may occur and that the risk of such transformation increases with the age of the tumour (the preoperative duration of the tumour). A microspectrophotometric DNA analysis shows a difference in the DNA content between morphologically benign pleomorphic adenomas with short and with long preoperative duration of the tumour. As cells from pleomorphic adenomas with a short preoperative duration contain the same diploid DNA value as normal parotid gland cells the pleomorphic adenomas with a longer preoperative duration are characterized by the occurrence of a small fraction of tetraploid cells in the population. This small fraction of tetraploid cells may be of great importance for the development of malignancy in these tumours as it was found that the cases of carcinoma in pleomorphic adenoma also have a tetraploid (or near tetraploid) fraction of cells. It is tempting to suggest that the tetraploid cells of the carcinoma in pleomorphic adenoma have originated from the tetraploid cells occurring in benign pleomorphic adenomas with prolonged clinical course.

The present clinical and microspectrophotometric study indicates the importance of an early operation even of the benign pleomorphic adenomas as the risk of malignant transformation of this type of tumour has been shown to increase with the preoperative duration of the tumour.

In recent years many of the debated problems concerning the malignancy of pleomorphic adenomas (mixed tumours) have been solved. Thus it has been shown that the concept of semimalignancy lacks justification and that the incidence of malignancy in pleomorphic adenoma is very

low—about 1–3% in large materials histologically classified according to modern nomenclature (Beahrs et al, 1957, Ackerman & del Regato, 1962, Eneroth, 1964, Eneroth et al, 1968).

It is still questioned, however, whether certain pleomorphic adenomas are primarily malignant or whether they become malignant at a later stage owing to a malignant transformation in a primarily benign pleomorphic adenoma. Evans & Cruickshank (1970) pointed out that if primary malignant mixed tumours (pleomorphic adenomas) exist at all, they must be exceedingly rare and that there is no question that a malignant transformation of an initially benign tumour can occur. The demonstration of a carcinomatous component in a benign pleomorphic adenoma and the demonstrated lack of pleomorphic adenoma structures in the metastases of these tumours argue in favour of the development of a carcinomatous component within the otherwise benign tumour (Eneroth et al, 1968). It has been discussed (Eneroth et al, 1968) whether this malignant change is related to the age of the tumour (preoperative duration of the tumour). This assumption, however, has not yet been satisfactorily proved. The reason is that malignancy in pleomorphic adenomas is very rare and therefore there is a lack of tumour materials of such a size that reliable conclusions concerning this question can be drawn.

Table 1 Case material microspectrophotometrically analysed

Histological features	Case no	Preoperative duration of the tumour (years)	Patients	
			Age (years)	Sex
Pleomorphic adenoma	1	2 12	39	M
Pleomorphic adenoma	2	1 12	52	M
Pleomorphic adenoma	3	6 12	33	M
Pleomorphic adenoma	4	5	52	F
Pleomorphic adenoma	5	5	36	F
Pleomorphic adenoma	6	6	54	F
Carcinoma in pleomorphic adenoma	7	16	69	M
Carcinoma in pleomorphic adenoma	8	15	59	M

In order to ascertain whether the malignant transformation in a pleomorphic adenoma is dependent on the preoperative duration a very large material of parotid tumours has been studied clinically and cells from pleomorphic adenomas with and without malignancy have been analysed microspectrophotometrically.

The importance of the time factor for malignant transformation in a benign pleomorphic adenoma can perhaps be elucidated by a microspectrophotometric DNA analysis, as increased nuclear DNA content has been shown to be a characteristic feature of malignant salivary gland tumour cells (Eneroth & Zetterberg, 1974).

For study of the influence of time on the nuclear DNA content in benign pleomorphic adenomas, microspectrophotometric DNA analyses were performed on cells from pleomorphic adenomas with different preoperative durations of the tumour and compared with the DNA values of cells from carcinoma in pleomorphic adenomas. The reason for investigating the nuclear DNA content in pleomorphic adenomas with various preoperative durations of the tumour was the possibility to find a difference of nuclear DNA content between cells from tumours with short and long preoperative duration—a difference which could reveal an early stage of malignant transformation of the tumour before any histological features of malignancy can be seen.

MATERIAL AND METHODS

The clinical staff was based on 623 pleomorphic adenomas of the parotid gland with or without malignancy, treated at the Department of Otolaryngology, Karolinska Sjukhuset, during the period 1930–1964. Forty patients with carcinoma in pleomorphic adenoma registered and treated at Radiumhemmet, Karolinska Sjukhuset, during the period 1923–1972 have also been studied. About 95% of the patients underwent regular follow-up examinations for 5–40 years.

The microspectrophotometric study was based on 8 pleomorphic adenomas with or without malignancy, treated at the Department of Otolaryngology, Karolinska Sjukhuset, in the year 1971. The age of the tumour, defined as the preoperative duration of the tumour, was less than one year in 3 cases of pleomorphic adenoma, more than four years in 3 cases of pleomorphic adenoma, and more than 15 years in 2 cases of carcinoma in pleomorphic adenoma (Table 1).

Immediately after the surgical removal of the tumours, imprint preparations were made from the fresh cut surface through the tumour tissue. Haemacytometer glass slides were used. The imprint preparations were immediately fixed in a freshly prepared mixture of ethanol and acetone (1:1) for 30 minutes at room temperature and thereafter stored in a refrigerator (+4°C) until the staining was performed.

For the microscopic examination of tumours,

Table II *The relation between the preoperative duration of the tumour and the incidence of malignancy in pleomorphic adenomas treated 1950-64*

Histological features	Preoperative duration of the tumour (Years)				Total no of cases
	0-4	5-9	10-14	>15	
Pleomorphic adenoma	430	80	48	48	606
Carcinoma in pleomorphic adenoma	7	2	3	5	17
Total	437	82	51	53	623
Incidence of malignancy (per cent)	1.6	2.4	5.9	9.4	

routine histopathological techniques were employed

The DNA content of individual cell nuclei was determined after Feulgen staining by absorption measurements in a rapid scanning microspectrophotometer at 546 nm (Lomakka, 1965, Caspersson & Lomakka, 1970). The optimal condition of the acid hydrolysis in the Feulgen staining procedure was used as described by Eneroth & Zetterberg (1974).

Freshly prepared human lymphocytes from peripheral blood were used as control cells of the staining procedure on each occasion. All measured values were expressed in relation to each staining control, which was given the value 2c, denoting the normal diploid DNA content.

Stromal cells with elongated nuclei, lymphocytes and granulocytes could relatively easily be cognized in Feulgen-stained preparations and were not included in the measurements.

RESULTS

Clinical study

During the period 1950-64 the age of the tumour at the time of the histological diagnosis, i.e. the interval between the first symptom of the tumour and the operation (the preoperative duration), was registered in 623 patients with parotid tumours reclassified as pleomorphic adenomas with or without malignancy. The histological features, the preoperative duration of the tumour as well as the influence of the duration on the incidence of malignancy in this material are shown in Table II. It is seen that malignant histological

structures appeared in 17 of the 623 tumours with pleomorphic adenoma features.

The incidence of malignancy increases progressively with the preoperative duration of the tumour, from 1.6% in tumours younger than 5 years to 9.4% in tumours older than 15 years.

As the incidence of malignancy in pleomorphic adenoma is very rare, the number of carcinomas in pleomorphic adenomas is comparatively small in relation to the large material of pleomorphic adenomas in the present series. Therefore we have also made calculations of the incidence of malignancy in a larger tumour material of carcinoma in pleomorphic adenoma treated at Radiumhemmet during the period 1923-72. This larger material of 40 patients with

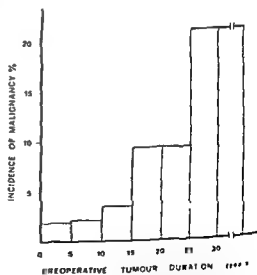


Fig. 1 Histogram showing the relation between the preoperative duration of the tumour and the incidence of malignancy in pleomorphic adenomas.

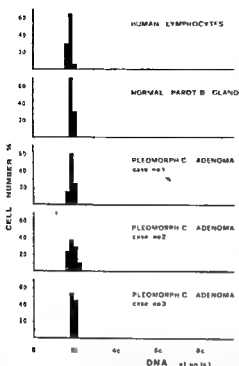


Fig 2 Histograms of nuclear DNA quantity (Feulgen-positive material) of individually analysed cell nuclei in the rapid scanning microspectrophotometer. Five different types of cell were analysed: human lymphocytes from peripheral blood (staining controls), cells from a normal parotid gland and cells from three cases of pleomorphic adenoma with a preoperative duration less than one year (Cases 1, 2 and 3). Each histogram is based on the microspectrophotometric analysis of between 50 and 75 randomly selected cells. The DNA values are expressed in relation to the staining control (human lymphocytes) which was given the value 2c.

carcinoma in pleomorphic adenomas has been related to the aforementioned material of pleomorphic adenomas from the period 1950-64. The results of the calculations of the incidence of malignancy in this large material as a function of the preoperative duration of the tumour are illustrated in a histogram (Fig 1). From this histogram it is seen still more emphatically that the incidence of malignancy in pleomorphic adenomas increases progressively with longer preoperative duration of the tumour.

The mean age of the 40 patients with carcinoma in pleomorphic adenoma was 52.2 years (range 23-83 years). There was no significant difference between the mean age of patients

whose tumours were of less than 5 years' duration (mean age 51.6 years) and those whose tumours were of more than 5 years' duration (mean age 52.6 years).

The observed preoperative duration of tumours ranged from a few months up to 55 years (mean 11.2 years). It should, however, be noted that the duration may have been longer than the patients themselves have stated.

Microspectrophotometric study of nuclear DNA content

In order to investigate whether the occasional clinical malignancy in aging pleomorphic adenoma is associated with any changes of the cytochemical properties of cells from older tumours,

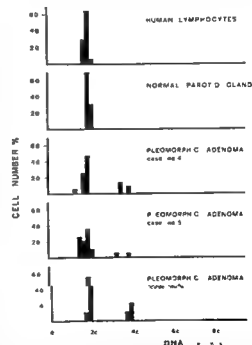


Fig 3 Histograms of nuclear DNA quantity (Feulgen-positive material) of individually analysed cell nuclei in the rapid scanning microspectrophotometer. Five different types of cell were analysed: human lymphocytes from peripheral blood (staining controls), cells from a normal parotid gland and cells from three cases of pleomorphic adenoma with a preoperative duration more than five years (Cases 4, 5 and 6). Each histogram is based on the microspectrophotometric analysis of between 50 and 75 randomly selected cells. The DNA values are expressed in relation to the staining control (human lymphocytes) which was given the value 2c.

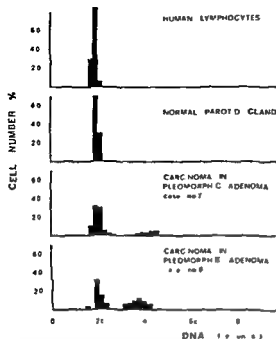


Fig 4 Histograms of nuclear DNA quantity (Feulgen positive material) of individually analysed cell nuclei in the rapid scanning microspectrophotometer. Four different types of cell were analysed: human lymphocytes from peripheral blood (staining controls), cells from a normal parotid gland and cells from two cases of carcinoma in pleomorphic adenoma with a preoperative duration of 15 and 16 years respectively (Cases 7 and 8). Each histogram is based on the microspectrophotometric analysis of between 50 and 75 randomly selected cells. The DNA values are expressed in relation to the staining control (human lymphocytes) which was given the value 2c.

nuclear DNA content was measured in individual pleomorphic adenoma cells of tumours with short and long preoperative duration. Fig 2 illustrates microspectrophotometric data of randomly selected Feulgen stained nuclei of imprint preparations from three cases of pleomorphic adenoma (cases 1, 2 and 3) with short preoperative duration (<one year) (cf Table I). As control cells of the Feulgen staining procedure human lymphocytes from peripheral blood were used. The average DNA content of the lymphocytes was given the value 2c denoting the normal diploid DNA content. Each measured value was related to its staining control, i.e. to the human lymphocytes stained together with the measured cell and expressed in the 2c lymphocyte units. As is evident from Fig 2,

all three cases of pleomorphic adenoma with short preoperative duration of the tumours (cases 1, 2 and 3) exhibited the same amount of DNA as normal parotid gland cells and lymphocytes namely the normal diploid content 2c. However, as is seen in Fig 3, in the three cases of pleomorphic adenoma with long preoperative duration, i.e. more than 5 years (cases 4, 5 and 6), an additional fraction of the cells varying from 10% to 30% of the population exhibited DNA values around 4c, i.e. twice the normal diploid value. This could either represent diploid cells in the premitotic phase (G2 phase) of the cell cycle or cells with a tetraploid (or near tetraploid) chromosome number. In the light of the very low mitotic index (less than 0.01%) which was observed in all these 6 cases of pleomorphic adenoma, it can be concluded that the cells with DNA content of 4c represent tetraploid (or near tetraploid) cells. It is therefore of great interest to note that the 2 cases of carcinoma developed in a pleomorphic adenoma (cases 7 and 8) also show a fraction of cells in the tetraploid region i.e. around 4c relative units of DNA (Fig 4). This fraction contains from 18% (case 7) to 45% (case 8) of the cells. Since these 2 cases of carcinoma in pleomorphic adenoma contain a mixture of relatively small benign adenoma cells and relatively large malignant carcinoma cells it is likely that the cells with nuclear DNA contents around 2c represent the adenoma cells and the cells with DNA values around 4c represent the carcinoma cells.

DISCUSSION

Although many of the problems concerning the malignancy of pleomorphic adenoma have now been answered, there is still a considerable uncertainty concerning the question whether a pleomorphic adenoma is a primarily benign type of tumour which can become malignant or whether carcinoma in pleomorphic adenoma is a primarily malignant type of tumour. The probability of malignant transformation of an initially benign tumour has been revealed by a

stological study of primary tumours and metastases (Moberger & Eneroth, 1968). A malignant transformation of a benign pleomorphic adenoma has also been suggested by the clinical features, as many of these cases had a long clinical course compared to other malignant salivary tumours. In a large series of parotid tumours (Eneroth et al., 1968) the mean interval between the first evidence of tumour and histological verification (the preoperative duration) ranged from 3.7 to 5.4 years in other types of malignant tumours, such as adenoid cystic carcinoma, acinic cell carcinoma and mucoepidermoid carcinoma. In carcinoma in pleomorphic adenoma this interval was 9 years. There are occasional reports in the literature of cases with very long preoperative duration of the tumour (>20 years) and with a short survival time after the operation, which indicates that malignant transformation of the pleomorphic adenoma cannot be regarded as improbable.

In an attempt to elucidate the hypothesis that the risk of malignant transformation in a pleomorphic adenoma increases with the preoperative duration of the tumour a comparison was made between this duration and the incidence of malignancy in pleomorphic adenoma in a large tumour material. The results from the present clinical study strongly indicate that malignant transformation of a pleomorphic adenoma is a function of time.

The earlier uncertainty in evaluating the malignancy of pleomorphic adenomas appears from the literature, since some authors divide these tumours into benign, semimalignant and malignant, others denote the whole group as solely benign, solely semimalignant or solely malignant. Correlation studies between histological and clinical features showing that this type of tumour should be divided into a benign and a malignant group (Eneroth, 1964) are also supported by the microspectrophotometric DNA analysis of the present study.

The microspectrophotometric data obtained in the present study show that cells from pleomorphic adenoma contain the diploid DNA value, i.e. these tumour cells have identical

DNA values to those of normal parotid gland cells, but they clearly differ from the cells of malignant salivary gland tumours described by Eneroth & Zetterberg (1974).

The results from the clinical study indicate the importance of the time factor for malignant transformation of a pleomorphic adenoma and in order to study whether the time factor also influences the nuclear DNA content of cells from pleomorphic adenomas, a microspectrophotometric DNA analysis was performed on cells from pleomorphic adenomas with different preoperative duration. This study showed that there was a difference in the DNA content between histologically benign pleomorphic adenomas with short and long preoperative duration. As cells from pleomorphic adenomas with a short preoperative duration (< one year) contain the diploid DNA value, the pleomorphic adenomas with a longer preoperative duration (> 5 years) are characterized by the occurrence of a small fraction of tetraploid cells (or near tetraploid cells) in the population.

However, the majority of the cells still have the diploid DNA value characteristic of normal parotid gland cells. Nevertheless this small fraction of tetraploid (or near tetraploid) cells may be of great importance for the development of malignancy in these tumours as discussed above. For it was found that the 2 cases of carcinoma in pleomorphic adenoma of the present study (cases 7 and 8) also have a tetraploid (or near tetraploid) fraction of cells, probably representing the malignant cells, in addition to the cells with normal diploid DNA values. These two malignant tumours (carcinoma in pleomorphic adenoma) thereby differ from other malignant salivary gland tumours which are characterized by the existence of aneuploid stemlines in the hyperdiploid region (Eneroth & Zetterberg, 1974). It is tempting to suggest that the tetraploid malignant cells have originated from the tetraploid cells of the benign pleomorphic adenomas, which may be more susceptible to malignant transformation than their counterparts. This is supported by an aberrant chromosome

the malignant transformation of cells, as indicated by Levan & Biesele (1958) in their experiments with embryonic mouse skin and cultures. Of particular relevance to the present work was their observation that the first cytological event in the transformation process was a shift in the chromosome number from diploidy to tetraploidy. Pleomorphic adenomas containing tetraploid cells might thus represent a potentially malignant tumour.

A wait-and see policy nevertheless exists regarding the treatment of parotid tumours, perhaps due to earlier statements in the literature that operation of parotid tumours is unnecessary (McFarland, 1933, 1942) and to the fact that the great majority—about 80% of parotid tumours—are benign (Eneroth, 1971).

The present clinical study has, however, shown that the risk of malignant transformation of a benign pleomorphic adenoma increases with the age of the tumour, and also the present microspectrophotometric analysis has shown nuclear DNA characteristics (tetraploid cells) in pleomorphic adenoma with a prolonged clinical course, which might be a preliminary stage of a malignant transformation.

Thus the results of the present clinical and microspectrophotometric studies indicate the importance of an early operation of all parotid tumours—even the benign pleomorphic adeno-

Zellen auftritt. Dieser Bruchteil an tetraploiden Zellen kann eine grosse Bedeutung in der Entwicklung des Malignitäts dieser Tumoren haben, da festgestellt werden konnte, dass in Fällen von Karzinomen in pleomorphen Adenomen auch ein gewisser Bruchteil an tetraploiden bzw. im tetraploiden Bereich liegenden Zellen vorliegt. Es ist daher naheliegend sich vorzustellen, dass die tetraploiden Zellen der Karzinome in pleomorphen Adenomen aus den tetraploiden Zellen hervorgegangen sind, die in benignen pleomorphen Adenomen in prolongiertem klinischen Verlauf auftreten.

Die vorliegende klinische und microspectrophotometrische Untersuchung unterstreicht, wie wichtig es auch benignen pleomorphen Adenomen frühzeitig zu operieren, da, wie gezeigt werden konnte, die Gefahr einer malignen Transformation dieses Tumortyps mit der präoperativen Dauer des Tumors zunimmt.

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ZUSAMMENFASSUNG

Wie die vorliegende klinische Untersuchung an Karzinomen in pleomorphen Adenomen, die auf dem grössten bisher in der Literatur beschriebenen Material basiert, entnehmen lässt, ist es sehr wahrscheinlich, dass bei einem ursprünglich benignen pleomorphen Adenom eine maligne Transformation eintreten kann und dass die Gefahr einer solchen Transformation mit dem Alter des Tumors, d. h. mit der präoperativen Dauer des Tumors, wächst. Wie die microspectrophotometrische DNS-Analyse zeigt, besteht ein Unterschied im DNS-Gehalt zwischen morphologisch benignen pleomorphen Adenomen von kurzer und von langer präoperativer Dauer des Tumors. Während Zellen von pleomorphen Adenomen von kurzer präoperativer Dauer den gleichen diploiden DNS-Betrag wie normale Parotiszellen enthalten, ist es für pleomorphe Adenome mit längerer präoperativer Dauer charakteristisch, dass bei ihnen auch ein gewisser (kleiner) Bruchteil an tetraploiden

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DECONGESTION OF NASAL MUCOUS MEMBRANES BY ORAL MEDICATION IN ACUTE RHINITIS

A Rhinomanometric Study to Demonstrate Synergism between Antihistamines and Adrenergic Substance

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Abstract Clinical trials of antihistamines combined with adrenergic substances showed a decongestive effect on nasal mucosal congestion due to acute rhinitis. Double blind technique combined with objective rhinomanometry over 9 hours in each individual trial showed complete absence of placebo effect, the results were therefore conclusive. Tests on 85 subjects with acute rhinitis as well as tests on 50 normal subjects with histamine provoked nasal blocking support the idea that an antihistamine and an adrenergic substance show synergism with regard to nasal decongestion. Of the drugs tested, clemastine combined with phenylpropanolamine gave the best result.

Decongestion of the nasal mucous membranes by oral medication is widely practised in patients with nasal allergy. Antihistamines or antihistamines combined with an adrenergic substance, assuming a synergism between the two types of drug, are the preparations most commonly used. It is practically unknown, however, whether this therapy is efficacious in congestion due to infection. The aim of the investigation was to ascertain whether oral medication might be of value in nasal blocking due to infection as well as in allergic congestion, and whether the synergism between an antihistamine and an adrenergic substance occurring in allergic nasal decongestion is a general rule. In an investigation published in 1964 Aschan demonstrated synergism between antihistamine and adrenergic substance in histamine provoked nasal congestion. This was the reason for including similar experiments in the present investigation for purpose of comparison.

MATERIAL AND METHODS

Altogether 85 subjects with nasal blocking due to acute rhinitis were included in the first part of the investigation. The tests were performed 2-3 days after the onset of the illness, and only individuals free from complications were included. The tests took place at a time of year when pollen allergy could be excluded as a source of error.

For the histamine provoked nasal blocking a total of 50 apparently healthy subjects were selected. Some took part in two experiments, the second after an interval of at least 4 weeks.

The nasal patency was measured by repeated rhinomanometric recordings, using the method described by Aschan et al (1956 and 1958). Recordings were made at intervals of 30-50 min over a period of 9 hours, and it was possible to trace the patency and changes in patency fairly well (see Figs 1 and 2). At each observation the airflow was recorded and calibrated to litres per min at a pressure gradient of 10 mm of water between the nostrils and oropharynx. For this pressure gradient an airflow of 30-40 litres per min may be regarded as normal. A lesser airflow indicates nasal congestion.

Double blind investigations were carried out for all drugs tested, and the key mixing list was opened and the rhinomanometric findings only evaluated.

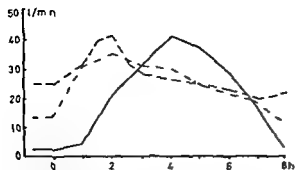


Fig. 1 Rhinomanometric changes in nasal patency in 3 subjects with nasal blocking due to acute rhinitis. Note the variations in patency before drug administration at 0. All test with active drug.

In the series with acute rhinitis the degree of nasal blocking before drug administration varied greatly from mild blocking to almost complete blocking of the nose, as is shown by the three positive experiments in Fig. 1. For this reason the final evaluation of an experiment was simplified to either positive as in Fig. 1, or negative. With complete or nearly complete blocking the rhinomanometric method does not work. If, for example, a subject with initial rhinomanometric values as in the lower curve in Fig. 1, did not respond, the rhinomanometric assessment ought to have been '0', but might of course also have been 'negative'. Because the double blind technique was used, such individual experiments had to be included.

Concerning the histamine experiments, each provocation involved the spraying of 1.5–2 ml

histamine (1:1000) into each nostril. An initial provocation served as individual control. The subsequent histamine provocations after drug administration were performed to ascertain how long the drug action, if any, persisted. In the experiments in Fig. 2, the dotted line represents a negative rhinomanometric finding; the unbroken line a positive result. In fact both experiments were performed on one and the same subject after an interval of several months and could be termed a double blind cross-over experiment. The first control histamine provocation in the two experiments in Fig. 2 also illustrates the reproducibility in the rhinomanometric method.

RESULTS

In an initial series 30 subjects with acute rhinitis were tested with '5031' (=15 mg Δ hydroxy ethyl promethazine chloride + ephedrine 10 mg). The results are shown in Table I.

A positive rhinomanometric result was obtained in 10 out of 15 tests using the active drug. No placebo effect whatsoever was observed or recorded by this objective method of assessment. The significance of the 10 out of 15 positive findings of nasal decongestion in acute rhinitis using 5031, compared with the placebo group must therefore be regarded as very high and conclusive.

In a second series (Table II) consisting of 15 subjects with acute rhinitis another antihista-

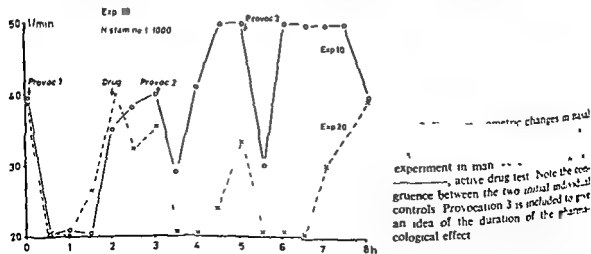


Table III *HSP 525 II Acute rhinitis*

Patient number	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
Judgement of rhinomanometric recording	+	+	-	+	-	-	+	-	+	-	+	+	-	-	-	-	+	-	-	-
Placebo			x			x							x		x					
HS 592				x	x							x		x						x
HSP 525 II		x					x		x		x									x
PPA	=							x		x						x	x			

brought to coincide more closely with that of clemastine

The final series of tests on acute rhinitis consisted of 20 subjects. The results are shown in Table IV, where HS 592 stands for clemastine 10 mg, PPA for phenyl propanolamine 50 mg retarded, and HSP 525 A for 50 mg PPA retarded with clemastine 10 mg. There was no placebo effect and no effect of antihistamine alone. The tests with both the combined preparation and the retarded phenylpropanolamine were all positive. The decongestive effect of the combined drug and of the adrenergic substance on nasal blocking due to acute rhinitis is unequivocal. Concerning the idea of synergism, the above-mentioned variation in patency in the subjects with rhinitis (see Fig. 1) and the small number of subjects in this series do not permit quantitative comparisons between HSP 525 A and PPA retarded. However, there is no evidence against the hypothesis of synergism.

Concerning the histamine experiments, the assessment of each individual experiment is explained in Fig. 2. Three series were run, large-

ly following the pattern of the experiments on acute rhinitis.

In the first series 10 subjects were tested with placebo and with HSP 525 I (clemastine 10 mg with 50 mg phenylpropanolamine). There was no placebo effect, and the active drug was positive in all 5 cases.

In a second series of 20 subjects HS 592 (clemastine 10 mg), PPA (30 mg phenylpropanolamine), and HSP 525 II were tested. There was no placebo effect. The antihistamine alone was positive in one out of 5, PPA positive in 5 out of 5, and HSP 525 II was positive in 3 out of 5 subjects.

In the last series of experiments on another 20 test persons the phenylpropanolamine used was '50 mg retarded', HS 592 and HSP 525 A, as in Table III above. Again as throughout the whole investigation, there was no placebo effect. With the antihistamine alone one test out of 5 was positive, with phenylpropanolamine retarded 4 out of 5 were positive, and for the antihistamine combined with the retarded phenylpropanolamine all five were positive.

Table IV *HSP 525 A Acute rhinitis*

Patient number	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
Judgement of rhinomanometric recording									-	+	-	+	-	-	+		+	+	-	
Placebo													x							
HS 592										x				x						x
HSP 525 A																				
PPA												x			x		x			

The histamine series thus supported the hypothesis of synergism between antihistamine and adrenergic substance with regard to nasal decongestion, even with this type of experimental nasal blocking in man. As can be seen from the typical experiment illustrated in Fig. 2, the active drug seemed to be effective for at least $5\frac{1}{2}$ hours after administration.

DISCUSSION

In clinical praxis nose drops are used for decongestion of the nasal mucous membranes no matter what the cause of the congestion. Topical administration of drugs has some drawbacks, however. Nose drops used for more than one week often cause remote reactions, and the patient becomes 'hooked' on his nose drops. This situation has not yet been reported in connexion with oral medication. Further, with topical administration, drugs producing maximum therapeutical responses on the nano- or microgram blood level must be given in concentrations as high as 5 to 10 mg/ml. As both nose drops and drugs given by mouth to produce nasal decongestion will act via the nasal blood vessels it would seem preferable to use the oral route, especially for long-term treatment. In clinical praxis it is as a rule desirable also to achieve decongestion of adjoining mucous membranes derived from the upper airways—the mucosa within the sinuses and their ostia, the Eustachian tube, and the middle ear. The chances of reaching these structures must be larger if the drug is distributed via the blood stream than if it is administered locally. This is particularly important when blocking is due to inflammatory causes.

Clinical testing in man has several shortcomings. The subjects with nasal blocking due to acute rhinitis were very carefully selected and represent only one type of inflammatory nasal blocking. The variations in degree of nasal blocking before drug administration represent another source of error; however, with the complete absence of placebo effects, the results must be regarded as conclusive. The drug combina-

tion marked 5031 had a decongestive effect in 10 out of 15 tests. The combination clemastine with phenylpropanolamine showed still better results, with 14 positive tests of 15, disregarding some galenic differences in the composition of the drug tested (HSP 525 I, HSP 525 II, and HSP 525 A). The significance of the results is proved by the thirty placebo tests, all of which were negative. The objective rhinomanometric technique used here shows that oral administration is the method of choice for obtaining nasal decongestion even when the nasal obstruction is due to inflammation.

A series of studies by Jackson (1971), Bentley & Jackson (1970) and Hall & Jackson (1968), on nasal patency, the sinuses, and the Eustachian tube in dogs, indicate that the mucous membranes in the different organs behave rather similarly from a pharmacological point of view. From their experiments it is evident that the nasal blood flow is regulated via nervous and pharmacological pathways. Dahlström & Fuxe (1965) have demonstrated the adrenergic innervation of the nasal blood vessels. They have also shown that no adrenergic fibres are present round the glands of the nasal mucosa in their animals, an interesting fact often neglected in connexion with nasal patency. Cauna & Hinderer (1969) have demonstrated by electron microscopy a dense innervation of the blood vessels in the human nasal mucosa. All the investigations quoted are of interest because they in different ways indicate that the decongestion obtained in the nasal mucosa by oral medication also occurs in adjoining structures, thus extending the indications for the use of such drugs.

The synergism between antihistamine and adrenergic substances in allergic nasal congestion is documented (McLaurin et al (1961), Pullen & Montgomery (1963), Aschan (1964), and others). Bentley & Jackson (1970) in experiments on dogs found a synergistic effect of several antihistamines and epinephrine upon histamine-provoked nasal blocking. The results of the experiments on acute rhinitis and of the experiments with histamine-provoked nasal congestion in man support the idea. The reason

clemastine in greater detail in this investigation was that it is known to produce little or no sedative effect. Phenylpropanolamine was chosen because of certain undesirable side-effects of ephedrine, particularly in elderly subjects.

The value of rhinomanometry in investigations of this type must be strongly emphasized. The reproducibility of the individual controls in the histamine provocation shown in Fig. 2 is one way of illustrating this. The results of Büsser & Schübl (1973), who used a somewhat different technique, provide further confirmation (see also Bühlmann, 1973). The complete absence of placebo effect in the double-blind series is further proof that rhinomanometry is the method of choice in clinical trials of this type.

ZUSAMMENFASSUNG

Klinische Versuche mit Antihistaminika in Kombination mit adrenergischen Substanzen ergaben einen dekonjestiven Effekt bei Schwellung der Nasenschleimhaut aufgrund akuter Rhinitis. Doppelblindtechnik verbunden mit objektiver Rhinomanometrie über einen Zeitraum von 9 Stunden bei jedem einzelnen Versuch zeigte das vollständige Ausbleiben des Placebo-effekts, die Resultate erwiesen sich daher als unverlässig. Versuche, durchgeführt an 85 Patienten mit akuter Rhinitis, sowie Versuche mit 50 normalen Versuchspersonen mit histamin-provozierter Blockade der Nase unterstützten die Annahme, dass Antihistamin zusammen mit einer adrenergischen Substanz Synergismus mit Wirkung auf die Dekongestion zeigt. Von den untersuchten Präparaten erzielte man die besten Ergebnisse mit einer Kombination von Clemastine 1,0 mg und Phenylpropanolamine 40 mg.

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ULTRASTRUCTURE OF THE EPITHELIUM IN ATROPHIC RHINITIS

Scanning Electron Microscopic Studies

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(Received October 29, 1973)

Abstract Biopsies from nasal mucosa of patients with atrophic rhinitis were studied in the scanning electron microscope. A keratinized squamous epithelium, resembling skin, was seen over large areas. Desquamation of horny cells is pronounced and abnormal, with large desquamated scales forming the basis for the well known crusts. Openings from submucosal glands and goblet cells are very infrequent. The small amount of mucus is dehydrated in situ, because of the lack of cilia.

The great depth of focus of scanning electron microscopy (SEM), its wide range of magnifications, and relatively great resolving power, make it a logical tool for the observation of the delicate structures of the surface of the human nasal mucosa. Within the last few years, several papers have appeared dealing with SEM studies of the normal nasal mucosa and the nasal mucosa from patients with perennial rhinitis (Lenz, 1972a, b, 1973, Mygind & Breilau, 1973, 1974). In addition Lenz (1972c) studied the squamous epithelium in the anterior part of the nasal cavity and described the normal desquamation process of the epithelial cells. In the present study the fine-structures of the horny cells and their spatial relationship within the squamous epithelium in atrophic rhinitis have been investigated. An attempt has been made to correlate these observations with information available from transmission electron microscopic investigations (Mygind et al., 1974).

MATERIAL AND METHODS

Six adult patients with pronounced atrophic rhinitis were examined. For a longer period of time they had all suffered from crust-formation in the nose and they had only been treated by conservative means. Biopsies of a size of 1½–3 mm were taken with a small forceps without local anaesthesia. They were taken from the lower edge of the inferior turbinate ½–1 cm behind the anterior edge as well as from other places in the nasal cavity.

For comparison purposes, biopsies were also obtained from the anterior part of the nasal cavity, at the borderline to the skin, in 3 normal persons.

As glutaraldehyde causes epithelium to adhere to the forceps, the biopsies were loosened from the forceps in 0.9% sodium chloride and immediately thereafter transferred to a cold 5% solution of glutaraldehyde, buffered with 0.03 M sodium cacodylate (pH 7.4). After 24 hours fixation the biopsies were stored in the buffer. After dehydration in ethyl alcohol the specimens were transferred to benzene. After freeze drying as described by Norrevang & Wingstrand (1970), the specimens were mounted on stubs, and a conduction coat of gold was deposited in vacuum. The specimens were studied in a Stereoscan S2.



Fig 1 Low-power magnification of nasal biopsy from patient with atrophic rhinitis. The surface of the mucous membrane is covered by irregular scales ($\times 42$)

RESULTS

The surface-topography of the epithelium is revealed in considerable detail in the scanning microscope, which also gives a striking three-dimensional appearance. At low power magnification the atrophic mucosa looks highly abnormal, with the appearance of "reptile skin" with numerous scales, irregularly arranged and of unequal size (Fig 1). At higher magnification one can see horny cells desquamate in the same manner as the horny cells in the anterior part of the nasal cavity, i.e. by initial elevation of the peripheral part of the cell (Fig 2). However, in the atrophic mucosa it is much more common to see several cells loosen *in toto*, forming large scales (Fig 3) or irregular dome-like shapes (Fig. 4). This suggests that the cells are quite flexible. Even though the horny cells are ex-

tremely thin, no examples of torn or fragmented cells were seen.

The horny cells in the skin of the anterior part of the normal nasal cavity are polygonal or hexagonal in shape and have smooth, straight intercellular junctions (Fig 5). One frequently encounters the junction of three cells at the corners of the contiguous hexagons. The cell-surface is not smooth, but covered by long, branching folds.

Whereas the picture of the normal nasal mucosa is quite different from this (Mygind & Bretlau, 1973, 1974), the atrophic nasal mucosa even in the posterior part of the nose has several similarities to the normal skin; only minor differences are seen. The horny cells in the atrophic mucosa may be polygonal in shape as the cells in the skin, but they are often more rounded, and it may be difficult to discern the cell borders

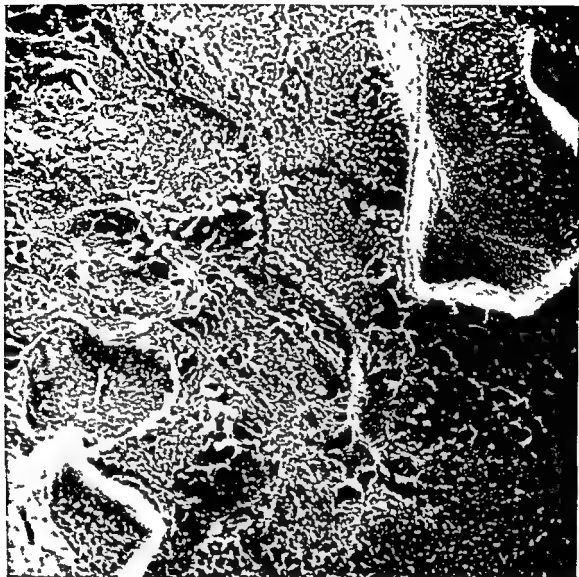


Fig 2 Surface of the atrophic mucous membrane. Medium power magnification. In the upper right corner is seen a horny cell with elevation of its peripheral part,

i.e. a normal desquamating process. The surface-structures of the cells are scattered, short, microvillous like projections ($\times 1800$).

(Fig 2) In contrast to the horny cells in the normal skin where long branching folds are the dominant surface structure the cell surfaces of the atrophic mucosa are mostly covered by short microvillous like projections (Fig 2). These projections may be remnants of desmosomes. On the whole the surface-structures of the atrophic mucosa are much shorter and more scattered than in the normal mucosa (Lenz, 1972a

Mygind & Bretlau 1973). Actual microvilli ($1.2 \mu\text{m}$ long structures) are not seen in the squamous epithelium.

In the specimens investigated from patients with atrophic rhinitis the dominant type of epithelium was a horny squamous epithelium, found in 4 out of 5 patients. In one biopsy, mally looking cilia were seen. In nearly half biopsies areas were observed of



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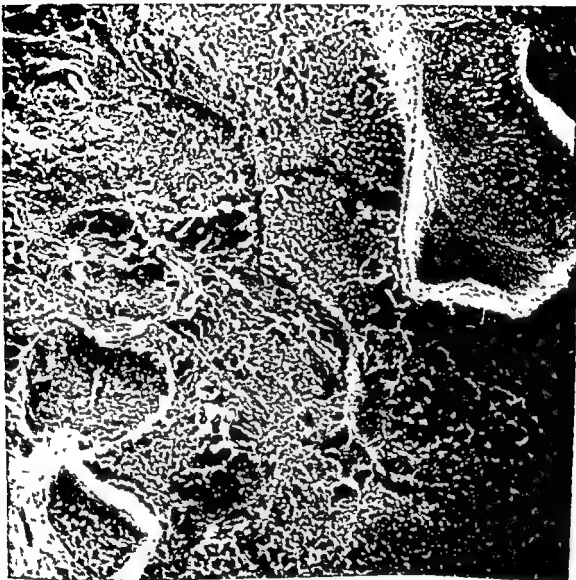


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Mygind & Bretlau, 1973). Actual microvilli (1–2 μm long structures) are not seen in the squamous epithelium.

In the specimens investigated from patients with atrophic rhinitis the dominant type of epithelium was a horny squamous epithelium found in 4 out of 6 patients. In one biopsy, normally looking cilia were seen. In nearly all biopsies, areas were observed of an



Fig 3 Abnormal desquamation of horny cells forming large scales ($\times 240$)

epithelial type, i.e. a non keratinized epithelium without cilia and actual microvilli. In these areas a single goblet cell was seen with its highly abnormal secretion being expelled from the cell but still in contact with the empty hole (Fig 6). The secretion looks dehydrated and viscid. Apart from this the surface was not covered by secretion. The polyp-like cytoplasmic protuberances from the epithelial cells, often found in normal nasal mucosa (Mygind & Bretlau 1974), are seldom observed in the atrophic specimens. The same is the case with the openings from submucosal glands.

DISCUSSION

As shown earlier (Mygind & Bretlau 1973) it is possible to use biopsies for SEM studies if they are treated with extreme caution. This offers new possibilities when examining the surface topography of different parts of the human respiratory tract under normal conditions, as well as in respiratory tract diseases. However, the technique for the preparation of biological specimens for SEM is still under development and caution in interpreting the results is necessary. The methods used for preparation of the specimens in the present study seem suitable as they produced results comparable to the pre-



Fig. 4 Abnormal desquamation of horny cells forming regular dome like shapes ($\times 1\ 000$)

ure seen with the transmission electron microscope. However, artefacts are inevitable in SEM. For example the microvillous like projections may appear somewhat enlarged, because of the gold coating, as the thickness of the gold layer may be of the same size as the projections themselves.

Extensive light-microscopic studies of atrophic rhinitis have been carried out by Holopainen (1967) who also observed keratinized squamous epithelium in some biopsies from different locations in the nasal cavity. In addition he showed that the squamous epithelium does not cover the total nasal cavity, but appears in islands. This is in accordance with our results. The occurrence of a horny squamous epithelium is very characteristic for atrophic rhinitis as it was

found in only 4 out of 100 biopsies from the nasal cavity in patients with perennial rhinitis and in normal persons (unpublished data).

The surface-structures in atrophic rhinitis, i.e. the microvillous like projections, are shorter and more scattered than the microvilli and cilia in the normal mucosa. This decrease in the surface area of the atrophic mucosa renders it less capable of holding moisture, resulting in a severely impaired ability to resist dehydration. When the epithelial surface is dry, the secretion from the scattered goblet cells is dehydrated *in situ*, as it is not transported away because of the lack of cilia. The same is the case with the desquamated cells, which as large scales form the basis for the well known crusts. Therefore, it seems that the primary feature in the crust formation



Fig. 3. Abnormal desquamation of horny cells forming large scales ($\times 240$).

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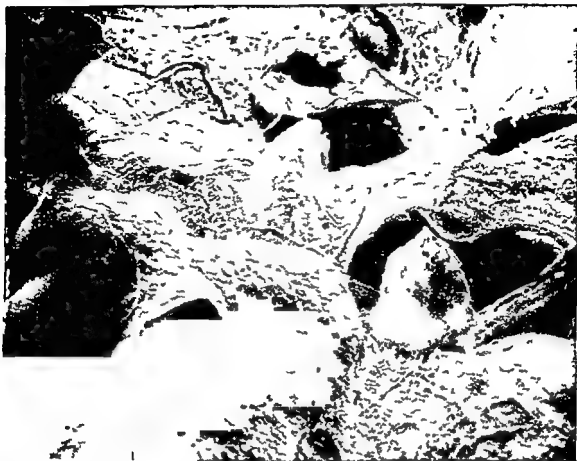


Fig 4 Abnormal desquamation of horny cells forming irregular dome like shapes ($\times 1000$)

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Fig 5 Surface of the skin in the anterior part of the nasal cavity, in a normal person. The surface structures are long branching folds (arrow) ($\times 2\,330$)

is a pathological pronounced desquamation and not an accumulation of neutrophilic leucocytes. The desquamation may be so intense that the surface resembles eczematous skin.

The similarities between the surface of the normal keratinized skin in the anterior part of the nasal cavity and the keratinized squamous epithelium of the metaplastic mucous membrane further back in the nose in atrophic rhinitis were striking. There were only minor differences between the shape and surface of the cells, but the horny cells in the atrophic mucosa desquamated in an intense and pathological way, not observed in the normal skin.

There was no consistent differences between the surface-structures on the top side and on the under side of the horny cells. This means that no proliferation of microvillous like surface structures takes place from the free cell-surface. The opposite is the case with the normal pseudostratified columnar epithelium, in which microvilli as well as cilia proliferate at the surface of the cells.

SEM studies of biopsies from normal persons and from patients with perennial rhinitis showed only minor differences (Mygind & Bretlau, 1973, 1974). It was impossible to decide whether a single biopsy originated from a normal



Fig 6 Expelled mucus from a goblet cell. Due to the lack of cilia, the mucus is not carried away and becomes dehydrated and viscid ($\times 4\,900$)

person or from a patient with perennial rhinitis, whereas in the case of atrophic rhinitis, nearly all biopsies appeared highly abnormal

ACKNOWLEDGMENT

The Cambo des Sciences, Paris, France, for the loan of the electron microscope.

ZUSAMMENFASSUNG

Biopsien der Nasenschleimhaut von Patienten mit atrophischer Rhinitis sind im Rasterelektronenmikroskop

untersucht worden. Es werden in weit verbreiteten Bereichen hautähnliche Plattenepithel festgestellt. Der Umfang von abgestossenen Schuppen, aus verhornten Zellen bestehend, ist ausgeprägt und abnorm, und dieselben bilden die Basis für die bekannten Schorfe. Es sind nur sehr wenige Ausmündungen von submukösen Drüsen und von Becherzellen vorhanden. Die kleine Mukusmenge trocknet in situ aufgrund von Mangel an Zilien aus.

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Table III *HSP 525 II Acute rhinitis*

Patient number	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
Judgement of rhinomanometric recording	+	+	-	+	-	-	+	-	+	-	+	+	-	-	-	-	+	-	-	+
Placebo			✓			✓							✓		×		✓			
HS 592				×	✓							✓		✓					✓	
HSP 525 II		✓					✓		✓		×									✓
PPA	✓							×		✓						×	×			

brought to coincide more closely with that of clemastine

The final series of tests on acute rhinitis consisted of 20 subjects. The results are shown in Table IV, where HS 592 stands for clemastine 10 mg, PPA for phenyl propanolamine 50 mg retarded, and HSP 525 A for 50 mg PPA retarded with clemastine 10 mg. There was no placebo effect and no effect of antihistamine alone. The tests with both the combined preparation and the retarded phenylpropanolamine were all positive. The decongestive effect of the combined drug and of the adrenergic substance on nasal blocking due to acute rhinitis is unequivocal. Concerning the idea of synergism, the above-mentioned variation in patency in the subjects with rhinitis (see Fig. 1) and the small number of subjects in this series do not permit quantitative comparisons between HSP 525 A and PPA retarded. However, there is no evidence against the hypothesis of synergism.

Concerning the histamine experiments, the assessment of each individual experiment is explained in Fig. 2. Three series were run, large

ly following the pattern of the experiments on acute rhinitis.

In the first series 10 subjects were tested with placebo and with HSP 525 I (clemastine 10 mg with 50 mg phenylpropanolamine). There was no placebo effect, and the active drug was positive in all 5 cases.

In a second series of 20 subjects HS 592 (clemastine 10 mg) PPA (30 mg phenylpropanolamine), and HSP 525 II were tested. There was no placebo effect. The antihistamine alone was positive in one out of 5, PPA positive in 4 out of 5, and HSP 525 II was positive in 3 out of 5 subjects.

In the last series of experiments on another 20 test persons the phenylpropanolamine used was '50 mg retarded', HS 592 and HSP 525 A as in Table III above. Again, as throughout the whole investigation, there was no placebo effect. With the antihistamine alone one test out of 5 was positive, with phenylpropanolamine retarded 4 out of 5 were positive, and for the antihistamine combined with the retarded phenylpropanolamine all five were positive.

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Patient number	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
Judgement of rhinomanometric recording	+	+				+	+	-	-	+		+	-	-	+	+	+	+	-	-
Placebo								×					✓							×
HS 592										✓				×					×	
HSP 525 A							✓			×					✓		✓	×		
PPA												×			✓		✓			

The histamine series thus supported the hypothesis of synergism between antihistamine and adrenergic substance with regard to nasal decongestion, even with this type of experimental nasal blocking in man. As can be seen from the typical experiment illustrated in Fig. 2, the active drug seemed to be effective for at least 5½ hours after administration.

DISCUSSION

In clinical praxis nose drops are used for decongestion of the nasal mucous membranes no matter what the cause of the congestion. Topical administration of drugs has some drawbacks, however. Nose drops used for more than one week often cause remote reactions, and the patient becomes 'hooked' on his nose drops. This situation has not yet been reported in connexion with oral medication. Further, with topical administration, drugs producing maximum therapeutical responses on the nano- or microgram blood level must be given in concentrations as high as 5 to 10 mg/ml. As both nose drops and drugs given by mouth to produce nasal decongestion will act via the nasal blood vessels it would seem preferable to use the oral route, especially for long-term treatment. In clinical praxis it is as a rule desirable also to achieve decongestion of adjoining mucous membranes derived from the upper airways—the mucosa within the sinuses and their ostia, the Eustachian tube, and the middle ear. The chances of reaching these structures must be larger if the drug is distributed via the blood stream than if it is administered locally. This is particularly important when blocking is due to inflammatory causes.

Clinical testing in man has several shortcomings. The subjects with nasal blocking due to acute rhinitis were very carefully selected, and represent only one type of inflammatory nasal blocking. The variations in degree of nasal blocking before drug administration represent another source of error. However, with the complete absence of placebo effects the results must be regarded as conclusive. The drug combina-

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Table III *HSP 525 II Acute rhinitis*

Patient number	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
Judgement of rhinomanometric recording	+	+	-	+	-	-	+	-	+	-	+	+		-		-	+	-	-	+
Placebo			x			x							x		x			x		
HS 592				x	x							x		x					x	
HSP 525 II		x					x		x		x									x
PPA	x							x		x						x	x			

brought to coincide more closely with that of clemastine

The final series of tests on acute rhinitis consisted of 20 subjects. The results are shown in Table IV, where HS 592 stands for clemastine 1.0 mg PPA for phenyl-propanolamine 50 mg retarded, and HSP 525 A for 50 mg PPA retarded with clemastine 1.0 mg. There was no placebo effect and no effect of antihistamine alone. The tests with both the combined preparation and the retarded phenylpropanolamine were all positive. The decongestive effect of the combined drug and of the adrenergic substance on nasal blocking due to acute rhinitis is unequivocal. Concerning the idea of synergism, the above-mentioned variation in patency in the subjects with rhinitis (see Fig. 1) and the small number of subjects in this series do not permit quantitative comparisons between HSP 525 A and PPA retarded. However, there is no evidence against the hypothesis of synergism.

Concerning the histamine experiments, the assessment of each individual experiment is explained in Fig. 2. Three series were run, large-

ly following the pattern of the experiments on acute rhinitis.

In the first series 10 subjects were tested with placebo and with HSP 525 I (clemastine 1.0 mg with 50 mg phenylpropanolamine). There was no placebo effect, and the active drug was positive in all 5 cases.

In a second series of 20 subjects HS 592 (clemastine 1.0 mg), PPA (30 mg phenylpropanolamine) and HSP 525 II were tested. There was no placebo effect. The antihistamine alone was positive in one out of 5, PPA positive in 5 out of 5, and HSP 525 II was positive in 3 out of 5 subjects.

In the last series of experiments on another 20 test persons the phenylpropanolamine used was '50 mg retarded', HS 592 and HSP 525 A as in Table III above. Again, as throughout the whole investigation, there was no placebo effect. With the antihistamine alone one test out of 5 was positive, with phenylpropanolamine retarded 4 out of 5 were positive, and for the antihistamine combined with the retarded phenylpropanolamine all five were positive.

Table IV *HSP 525 A Acute rhinitis*

Patient number	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	
Judgement of rhinomanometric recording		+	+	-	-	+	+	-	-	+	-	+	-			+	+	+	+	-	-
Placebo								x	x				x							x	y
HS 592					x						x			x							
HSP 525 A							x			x							x		x		
PPA			x			x						x				x		x			

The histamine series thus supported the hypothesis of synergism between antihistamine and adrenergic substance with regard to nasal decongestion, even with this type of experimental nasal blocking in man. As can be seen from the typical experiment illustrated in Fig. 2, the active drug seemed to be effective for at least 5½ hours after administration.

DISCUSSION

In clinical praxis nose drops are used for decongestion of the nasal mucous membranes no matter what the cause of the congestion. Topical administration of drugs has some drawbacks, however. Nose drops used for more than one week often cause remote reactions, and the patient becomes 'hooked' on his nose drops. This situation has not yet been reported in connexion with oral medication. Further, with topical administration, drugs producing maximum therapeutical responses on the nano- or microgram blood level must be given in concentrations as high as 5 to 10 mg/ml. As both nose drops and drugs given by mouth to produce nasal decongestion will act via the nasal blood vessels it would seem preferable to use the oral route, especially for long-term treatment. In clinical praxis it is as a rule desirable also to achieve decongestion of adjoining mucous membranes derived from the upper airways—the mucosa within the sinuses and their ostia, the Eustachian tube, and the middle ear. The chances of reaching these structures must be larger if the drug is distributed via the blood stream than if it is administered locally. This is particularly important when blocking is due to inflammatory causes.

Clinical testing in man has several shortcomings. The subjects with nasal blocking due to acute rhinitis were very carefully selected, and represent only one type of inflammatory nasal blocking. The variations in degree of nasal blocking before drug administration represent another source of error; however, with the complete absence of placebo effects, the results must be regarded as conclusive. The drug combina-

tion marked 5031 had a decongestive effect in 10 out of 15 tests. The combination clemastine with phenylpropanolamine showed still better results, with 14 positive tests of 15, disregarding some galenic differences in the composition of the drug tested (HSP 525 I, HSP 525 II, and HSP 525 A). The significance of the results is proved by the thirty placebo tests, all of which were negative. The objective rhinomanometric technique used here shows that oral administration is the method of choice for obtaining nasal decongestion even when the nasal obstruction is due to inflammation.

A series of studies by Jackson (1971), Bentley & Jackson (1970), and Hall & Jackson (1968), on nasal patency, the sinuses, and the Eustachian tube in dogs, indicate that the mucous membranes in the different organs behave rather similarly from a pharmacological point of view. From their experiments it is evident that the nasal blood flow is regulated via nervous and pharmacological pathways. Dahlström & Fuxe (1965) have demonstrated the adrenergic innervation of the nasal blood vessels. They have also shown that no adrenergic fibres are present round the glands of the nasal mucosa in their animals, an interesting fact often neglected in connexion with nasal patency. Cauna & Hinderer (1969) have demonstrated by electron microscopy a dense innervation of the blood vessels in the human nasal mucosa. All the investigations quoted are of interest because they in different ways indicate that the decongestion obtained in the nasal mucosa by oral medication also occurs in adjoining structures, thus extending the indications for the use of such drugs.

The synergism between antihistamine and adrenergic substances in allergic nasal congestion is documented (McLaurin et al (1961), Pullen & Montgomery (1963), Aschan (1964), and others). Bentley & Jackson (1970) in experiments on dogs found a synergistic effect of several antihistamines and epinephrine upon histamine-provoked nasal blocking. The results of the experiments on acute rhinitis and of the series with histamine provoked nasal congestion in man support the idea. The reason for stud-

clemastine in greater detail in this investigation was that it is known to produce little or no sedative effect. Phenylpropanolamine was chosen because of certain undesirable side-effects of ephedrine, particularly in elderly subjects.

The value of rhinomanometry in investigations of this type must be strongly emphasized. The reproducibility of the individual controls in the histamine provocation shown in Fig 2 is one way of illustrating this. The results of Büsser & Schibli (1973), who used a somewhat different technique, provide further confirmation (see also Bühlmann, 1973). The complete absence of placebo effect in the double-blind series is further proof that rhinomanometry is the method of choice in clinical trials of this type.

ZUSAMMENFASSUNG

Klinische Versuche mit Antihistaminika in Kombination mit adrenergischen Substanzen ergaben einen dekongestiven Effekt bei Schwellung der Nasenschleimhaut aufgrund akuter Rhinitis. Doppelblindtechnik verbunden mit objektiver Rhinomanometrie über einen Zeitraum von 9 Stunden bei jedem einzelnen Versuch zeigte das vollständige Ausbleiben des Placebo-effekts. Die Resultate erwiesen sich daher als unverlässlich. Versuche, durchgeführt an 85 Patienten mit akuter Rhinitis, sowie Versuche mit 50 normalen Versuchspersonen mit histamin-provozierter Blockade der Nase unterstützten die Annahme, dass Antihistamin zusammen mit einer adrenergischen Substanz Synergismus mit Wirkung auf die nasale Dekongestion zeigt. Von den untersuchten Präparaten erzielte man die besten Ergebnisse mit einer Kombination von Clemastine 10 mg und Phenylpropanolamine 50 mg.

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ULTRASTRUCTURE OF THE EPITHELIUM IN ATROPHIC RHINITIS

Scanning Electron Microscopic Studies

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Abstract Biopsies from nasal mucosa of patients with atrophic rhinitis were studied in the scanning electron microscope. A keratinized squamous epithelium, resembling skin, is seen over large areas. Desquamation of horny cells is pronounced and abnormal, with large desquamated scales forming the basis for the well known crusts. Openings from submucosal glands and goblet cells are very infrequent. The small amount of mucus is dehydrated in situ, because of the lack of cilia.

The great depth of focus of scanning electron microscopy (SEM), its wide range of magnifications, and relatively great resolving power, make it a logical tool for the observation of the delicate structures of the surface of the human nasal mucosa. Within the last few years, several papers have appeared dealing with SEM studies of the normal nasal mucosa and the nasal mucosa from patients with perennial rhinitis (Lenz, 1972a, b, 1973, Mygind & Bretlau, 1973, 1974). In addition Lenz (1972c) studied the squamous epithelium in the anterior part of the nasal cavity and described the normal desquamation process of the epithelial cells. In the present study the fine-structures of the horny cells and their spatial relationship within the squamous epithelium in atrophic rhinitis have been investigated. An attempt has been made to correlate these observations with information available from transmission electron microscopic investigations (Mygind et al., 1974).

MATERIAL AND METHODS

Six adult patients with pronounced atrophic rhinitis were examined. For a longer period of time they had all suffered from crust formation in the nose and they had only been treated by conservative means. Biopsies of a size of 1½-3 mm were taken with a small forceps without local anaesthesia. They were taken from the lower edge of the inferior turbinate ½-1 cm behind the anterior edge as well as from other places in the nasal cavity.

For comparison purposes, biopsies were also obtained from the anterior part of the nasal cavity, at the borderline to the skin, in 3 normal persons.

As glutaraldehyde causes epithelium to adhere to the forceps, the biopsies were loosened from the forceps in 0.9% sodium chloride and immediately thereafter transferred to a cold 5% solution of glutaraldehyde, buffered with a 0.03 M sodium cacodylate (pH 7.4). After 24 hours fixation the biopsies were stored in the buffer. After dehydration in ethyl alcohol the specimens were transferred to benzene. After freeze-drying as described by Nørrevang & Wingstrand (1970) the specimens were mounted on stubs and a conduction coat of gold was deposited in vacuum. The specimens were studied in a Cambridge Stereoscan S2.

clemastine in greater detail in this investigation was that it is known to produce little or no sedative effect. Phenylpropanolamine was chosen because of certain undesirable side-effects of ephedrine, particularly in elderly subjects.

The value of rhinomanometry in investigations of this type must be strongly emphasized. The reproducibility of the individual controls in the histamine provocation shown in Fig. 2 is one way of illustrating this. The results of Büsser & Schibli (1973), who used a somewhat different technique, provide further confirmation (see also Bühlmann, 1973). The complete absence of placebo effect in the double-blind series is further proof that rhinomanometry is the method of choice in clinical trials of this type.

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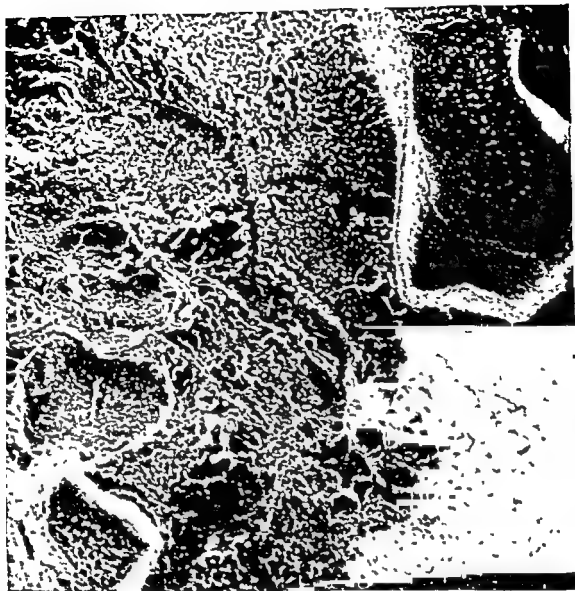


Fig 2 Surface of the atrophic mucous membrane. Medium-power magnification. In the upper right corner is seen a horny cell with elevation of its peripheral part,

i.e. a normal desquamation process. The surface-structures of the cells are scattered, short, microvillous like projections ($\times 1800$).

(Fig 2) In contrast to the horny cells in the normal skin, where long, branching folds are the dominant surface structure, the cell surfaces of the atrophic mucosa are mostly covered by short microvillous-like projections (Fig 2). These projections may be remnants of desmosomes. On the whole, the surface structures of the atrophic mucosa are much shorter and more scattered than in the normal mucosa (Lenz, 1972a,

Mygind & Bretlau, 1973). Actual microvilli (1–2 μm long structures) are not seen in the squamous epithelium.

In the specimens investigated from patients with atrophic rhinitis the dominant type of epithelium was a horny squamous epithelium, found in 4 out of 6 patients. In one biopsy, normally looking cilia were seen. In nearly half of the biopsies, areas were observed of an intermediate



Fig 1 Low power magnification of nasal biopsy from patient with atrophic rhinitis. The surface of the mucous membrane is covered by irregular scales ($\times 42$).

RESULTS

The surface-topography of the epithelium is revealed in considerable detail in the scanning microscope, which also gives a striking three dimensional appearance. At low power magnification the atrophic mucosa looks highly abnormal, with the appearance of "reptile skin" with numerous scales, irregularly arranged and of unequal size (Fig 1). At higher magnification one can see horny cells desquamate in the same manner as the horny cells in the anterior part of the nasal cavity, i.e. by initial elevation of the peripheral part of the cell (Fig 2). However, in the atrophic mucosa it is much more common to see several cells loosen *in toto*, forming large scales (Fig 3) or irregular dome-like shapes (Fig 4). This suggests that the cells are quite flexible. Even though the horny cells are ex-

tremely thin, no examples of torn or fragmented cells were seen.

The horny cells in the skin of the anterior part of the normal nasal cavity are polygonal or hexagonal in shape and have smooth straight intercellular junctions (Fig 5). One frequently encounters the junction of three cells at the corners of the contiguous hexagons. The cell surface is not smooth, but covered by long, branching folds.

Whereas the picture of the normal nasal mucosa is quite different from this (Mygind & Bretlau, 1973, 1974) the atrophic nasal mucosa even in the posterior part of the nose has several similarities to the normal skin. Only minor differences are seen. The horny cells in the atrophic mucosa may be polygonal in shape as the cells in the skin, but they are often more roundish and it may be difficult to discern the cell borders



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Fig 3 Abnormal desquamation of horny cells forming large scales ($\times 740$)

epithelial type i.e. a non keratinized epithelium without cilia and actual microvilli. In these areas a single goblet cell was seen with its highly abnormal secretion being expelled from the cell but still in contact with the empty hole (Fig 6). The secretion looks dehydrated and viscid. Apart from this the surface was not covered by secretion. The polyp like cytoplasmic protuberances from the epithelial cells often found in normal nasal mucosa (Mygind & Bretlau 1974) are seldom observed in the atrophic specimens. The same is the case with the openings from sub-mucosal glands.

DISCUSSION

As shown earlier (Mygind & Bretlau 1973) it is possible to use biopsies for SEM studies if they are treated with extreme caution. This offers new possibilities when examining the surface topography of different parts of the human respiratory tract under normal conditions as well as in respiratory tract diseases. However the technique for the preparation of biological specimens for SEM is still under development and caution in interpreting the results is necessary. The methods used for preparation of the specimens in the present study seem suitable as they produced results comparable to the p.c.



Fig. 4. Abnormal desquamation of horny cells forming regular, dome like shapes ($\times 1000$)

seen with the transmission electron microscope. However, artefacts are inevitable in EM. For example the microvillous-like projections may appear somewhat enlarged, because of the gold coating, as the thickness of the gold layer may be of the same size as the projections themselves.

Extensive light microscopic studies of atrophic rhinitis have been carried out by Holopainen (1967) who also observed keratinized squamous epithelium in some biopsies from different locations in the nasal cavity. In addition he showed that the squamous epithelium does not cover the total nasal cavity, but appears in islands. This is in accordance with our results. The occurrence of a horny squamous epithelium is very characteristic for atrophic rhinitis, as it was

found in only 4 out of 100 biopsies from the nasal cavity in patients with perennial rhinitis and in normal persons (unpublished data).

The surface structures in atrophic rhinitis, i.e. the microvillous like projections, are shorter and more scattered than the microvilli and cilia in the normal mucosa. This decrease in the surface area of the atrophic mucosa renders it less capable of holding moisture, resulting in a severely impaired ability to resist dehydration. When the epithelial surface is dry, the secretion from the scattered goblet cells is dehydrated *in situ*, as it is not transported away because of the lack of cilia. The same is the case with the desquamated cells, which as large scales form the basis for the well-known crusts. Therefore, it seems that the primary feature in the crust formation

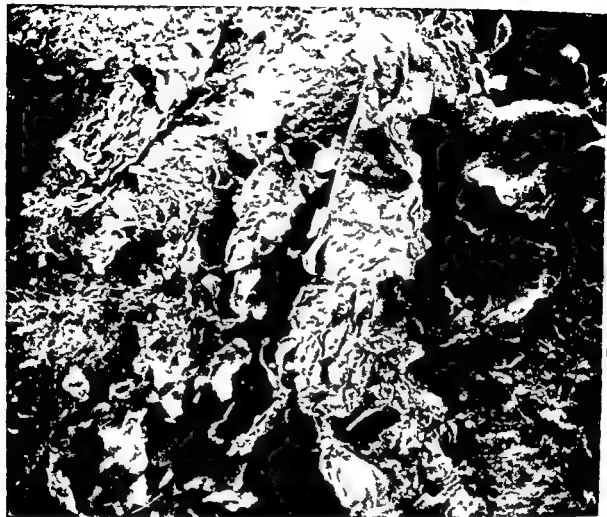


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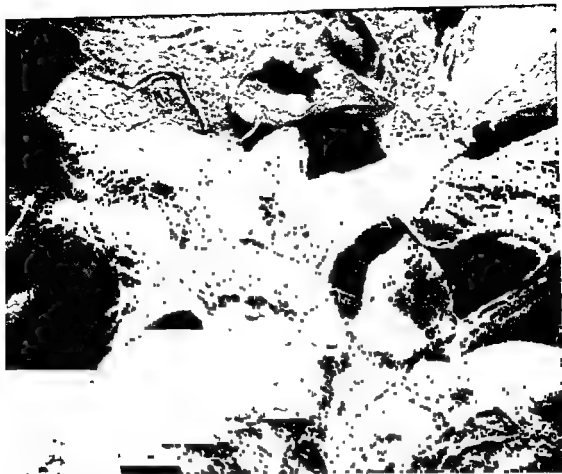


Fig. 4. Abnormal desquamation of horny cells forming angular, dome like shapes ($\times 1000$)

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Fig 3 Abnormal desquamation of horny cells forming large scales ($\times 240$)

epithelial type, i.e. a non-keratinized epithelium without cilia and actual microvilli. In these areas a single goblet cell was seen with its highly abnormal secretion being expelled from the cell, but still in contact with the empty hole (Fig. 6). The secretion looks dehydrated and viscid. Apart from this, the surface was not covered by secretion. The polyp-like cytoplasmic protuberances from the epithelial cells, often found in normal nasal mucosa (Mygind & Bretlau, 1974), are seldom observed in the atrophic specimens. The same is the case with the openings from sub-mucosal glands.

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Fig. 4 Abnormal desquamation of horny cells forming irregular, dome like shapes ($\times 1000$)

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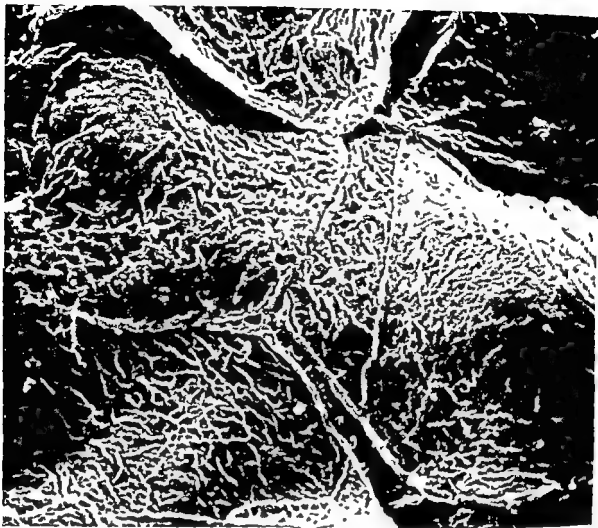


Fig 5 Surface of the skin in the anterior part of the nasal cavity in a normal person. The surface structures are long branching folds (arrow) ($\times 2\,330$)

is a pathological pronounced desquamation and not an accumulation of neutrophilic leucocytes. The desquamation may be so intense that the surface resembles eczematous skin.

The similarities between the surface of the normal keratinized skin in the anterior part of the nasal cavity and the keratinized squamous epithelium of the metaplasized mucous membrane further back in the nose in atrophic rhinitis were striking. There were only minor differences between the shape and surface of the cells, but the horny cells in the atrophic mucosa desquamated in an intense and pathological way, not observed in the normal skin.

There was no consistent differences between the surface structures on the top side and on the under side of the horny cells. This means that no proliferation of microvillous like surface-structures takes place from the free cell surface. The opposite is the case with the normal pseudostratified columnar epithelium in which microvilli as well as cilia proliferate at the surface of the cells.

EM studies of biopsies from normal persons and from patients with perennial rhinitis showed only minor differences (Mygind & Bretlau, 1973, 1974). It was impossible to decide whether a single biopsy originated from a normal

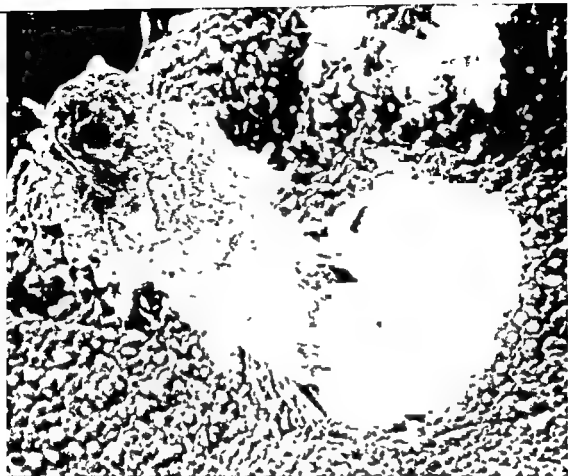


Fig. 6 Expelled mucus from a goblet cell. Due to the lack of cilia, the mucus is not carried away and becomes dehydrated and viscid ($\times 4900$).

erson or from a patient with perennial rhinitis, whereas in the case of atrophic rhinitis, nearly all biopsies appeared highly abnormal.

ACKNOWLEDGMENT

The Cambridge Stereoscan microscope was kindly placed

at our disposal by Dr. J. E. R. and Mr. Erik Jensenius for skilful technical assistance.

ZUSAMMENFASSUNG

Biopsien der Nasenschleimhaut von Patienten mit atrophischer Rhinitis sind im Rasterelektronenmikroskop

untersucht worden. Es werden in weit verbreiteten Bereichen hautähnliche Plattenepithelien festgestellt. Der Umfang von abgestoßenen Schuppen, aus verhornten Zellen bestehend, ist ausgeprägt und abnorm, und dieselben bilden die Basis für die bekannten Schorfe. Es sind nur sehr wenige Ausmündungen von submukösen Drüsen und von Becherzellen vorhanden. Die kleine Mukusmenge trocknet in situ aufgrund von Mangel an Zilien aus.

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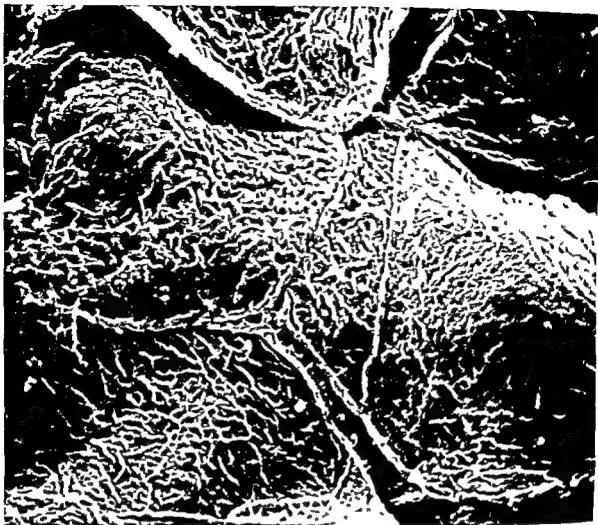


Fig 5 Surface of the skin in the anterior part of the nasal cavity, in a normal person. The surface structures are long branching folds (arrow) ($\times 2\,330$)

as a pathological pronounced desquamation and not an accumulation of neutrophilic leucocytes. The desquamation may be so intense that the surface resembles eczematous skin.

The similarities between the surface of the normal keratinized skin in the anterior part of the nasal cavity and the keratinized squamous epithelium of the metaplasized mucous membrane further back in the nose in atrophic rhinitis were striking. There were only minor differences between the shape and surface of the cells, but the horny cells in the atrophic mucosa desquamated in an intense and pathological way, not observed in the normal skin.

There was no consistent differences between the surface-structures on the top side and on the under side of the horny cells. This means that no proliferation of microvillous like surface structures takes place from the free cell surface. The opposite is the case with the normal pseudostratified columnar epithelium, in which microvilli as well as cilia proliferate at the surface of the cells.

SEM studies of biopsies from normal persons and from patients with perennial rhinitis showed only minor differences (Mygind & Bretlau, 1973, 1974). It was impossible to decide whether a single biopsy originated from a normal

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